EXHIBIT 1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	
Libutti *et al.*	
	Group Art Unit: 1633
Serial No. 10/031,008	
	Examiner: Burkhart, M.
Filed: May 6, 2002	
	Confirmation No. 3848
FOR: METHODS FOR TREATING TUMORS USING ANTIANGIOGENIC COMPOUNDS	

DECLARATION OF RENATA PASQUALINI UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

NEEDLE & ROSENBERG P.C.
Customer Number 36339

Sir:

I, Renata Pasqualini, a citizen of America, residing at 1914 West Gray #308, 77019, Houston, TX, declare that:

1. "I have read and understood the claims of the above referenced application and am familiar with the construct described therein."

2. I received my Ph.D. degree from the University of Sao Paulo, Brazil, and undertook postdoctoral training at Harvard Medical School and The Burnham Institute. I have been conducting cancer research since 1983 and am a co-author of over 100 publications relating to cancer research. I am currently the Buchanan and Seeger Professor of Medicine and Cancer Biology at The University of Texas M.D. Anderson Cancer Center. A copy of my Curriculum Vitae is attached as Exhibit A.

3. I have read and understood U.S. Patent No. 5,733,548 (Restifo et al.) and U.S. Patent No. 6,638,502 (Li et al.). I am familiar with the research of Restifo et al., for example, as described in U.S. Patent No. 5,733,584. I am also familiar with the research of Li et al., for example, as described in U.S. Patent No. 6,638,502. It is my belief that although Restifo et al. discloses administration of P1A tumor peptide (SEQ ID NO: 6 (9 amino acids)) linked to an adenoviral E19 signal sequence in order to induce an immune response and Li et al. discloses intratumoral administration of an adenoviral vector that expresses angiostatin, one of skill in the art would not have considered utilizing the delivery system of Restifo et al. with the antiangiogenic protein of Li et al. in order to arrive at the claimed compositions of the instant application. The work of Restifo et al. showed only that the adenoviral E19 signal sequence could be used for expressing a small peptide in order to induce an immunogenic response. This is not closely related to a construct that expresses a full-sized protein that has anti-angiogenic function. Although Restifo et al. makes the unsupported assertion that the E19 signal sequence can drive expression and secretion of a peptide from 5 to1000 amino acids in length, people of skill in the cancer therapy field did not view Restifo et al. as providing a reasonable expectation that an adenoviral E19 signal sequence could drive expression of an antiangiogenic protein that targets endothelial cells, and results in increased circulating levels of the antiangiogenic protein in order to treat tumors via systemic administration, particularly when the only example set forth by Restifo et al. is a small peptide.

Furthermore, Li et al. disclose unrelated (not adenoviral) signal sequences to direct the secretion of antiangiogenic proteins expressed from adenoviral vectors. In fact, these signal sequences are sequences that are naturally associated with the antiangiogenic proteins being expressed by the vectors of Li et al. For example, the urokinase signal sequence is utilized to effect secretion of urokinase. In another example, the plasminogen signal sequence is utilized to effect secretion of an N-terminal fragment of human plasminogen. Li et al. teach only signal sequences that allow secretion of the polypeptide with which they are associated in nature (i.e. urokinase signal sequence and urokinase or plasminogen signal sequence and an N-terminal fragment of plasminogen). This teaching is conceptually distinct from and not suggestive of the

idea that any signal sequence other than the signal sequence naturally associated with urokinase or plasminogen would result in the expression of these antiangiogenic molecules. It is even more surprising that a different signal sequence, for example, an adenoviral signal sequence, could be used to produce increased circulating levels of an antiangiogenic protein in order to treat tumors via systemic delivery as is shown in the present application.

I have also read and understand Griscelli et al. ("Angiostatin gene transfer: Inhibition of tumor growth *in vivo* by blockage of endothelial cell proliferation associated with a mitosis arrest" *PNAS* 95: 6367-6372, 1998). Similar to Li et al., Griscelli et al. teach an adenoviral vector that expresses the N-terminal fragment (amino acids 1-333) from human plasminogen operatively linked to the plasminogen signal sequence , i.e. the signal sequence that it is associated with in nature, and not an adenoviral signal sequence. The results obtained with the Griscelli et al. vector, wherein systemic administration of this vector resulted in decreased tumor establishment and growth, would not be considered to suggest the likelihood of a similar result when using an unrelated signal sequence, particularly a signal sequence not naturally associated with the expressed protein. Thus, one of skill in the art would not have thought to alter the vector of Griscelli et al. by utilizing an unrelated signal sequence, much less the adenoviral E19 signal sequence of Restifo et al. that was not naturally associated with any antiangiogenic protein and had only been utilized to secrete small peptides. This is because, there would have been no reasonable expectation that a nucleic acid encoding an antiangiogenic protein operatively linked to an adenoviral signal sequence would have worked at all, much less had properties that significantly advanced the practice of cancer therapy. There was no suggestion in the papers noted that the adenoviral E19 signal sequence-antiangiogenic protein construct would have the properties shown by Libutti et al.: 1) the ability to increase circulating levels of an antiangiogenic protein; and 2) the ability to treat tumors via systemic delivery.

4. I further declare that the results obtained by Libutti et al. with the claimed compositions have been lauded by others in the field as promising in the face of conflicting results with other anti-angiogenic therapies. For example, Dr. Judah Folkman, a renowned cancer researcher, when commenting on the paradoxical behavior of endostatin, noted that "...a

gene therapy experiment by Andrew Feldman and Steven Libutti at NCI did produce some promising results. Feldman and Libutti transplanted an endostatin gene into mouse liver tumor cells and implanted the cells into mice. As they reported in the *Journal of the National Cancer Institute* last year, the implants expressing the highest amounts of endostatin were most strongly inhibited from growing." (See Exhibit B, "Setbacks for Endostatin," *Science* 295:2198-2199 (2002)) In this reference, the mouse liver tumor cells were transduced with a construct comprising a recombinant nucleic acid encoding an antiangiogenic protein, i.e. endostatin, operatively linked to an adenovirus signal sequence inserted within a viral nucleic acid, i.e. a retroviral nucleic acid (See Exhibit C, Feldman et al. "Effects of Retroviral Endostatin Gene Transfer on Subcutaneous and Intraperitoneal Growth of Murine Tumors" *Journal of the National Cancer Institute* 93(13): 1014-1020 (2001)). Therefore, in addition to treating tumors systemically, the claimed compositions have the ability to treat tumors via *ex vivo* transduction of tumor cells.

Until the development of the claimed construct, nobody had been able to successfully treat tumors in both the systemic and implantation contexts. Furthermore, the circulating levels of endostatin achieved by Dr. Libutti were unprecedented, thus providing for the first time, evidence that a host can be utilized as a factory for production of sufficiently increased levels of an antiangiogenic polypeptide to effectively treat cancer patients. Therefore, it is my belief that the claimed compositions were a significant breakthrough in the field of cancer therapy.

4. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any patent issuing therefrom.

RENATA PASQUALINI

NOV 27, 2007
DATE

EXHIBIT A

CURRICULUM VITAE

NAME

Renata Pasqualini, Ph.D.

PRESENT TITLE AND AFFILIATION

Primary Appointment
Professor of Medicine
Helen Buchanan and Stanley Joseph Seeger Research Professorship
The University of Texas M. D. Anderson Cancer Center, Houston, Texas

Dual/Joint Appointment
Professor of Cancer Biology, Department of Cancer Biology

Distinguished Faculty, The University of Texas M. D. Anderson Cancer Center Trust

CITIZENSHIP AND VISA STATUS

Brazilian, Italian, American

HOME ADDRESS

1914 West Gray Avenue #308
Houston, TX 77019
Phone: (713) 528-9018

OFFICE ADDRESS

The University of Texas M. D. Anderson Cancer Center
1515 Holcombe Boulevard, Room T7.3970, Unit 1374
Houston, TX 77230-1439
Voice Mail: (713) 792-3872
Office: (713) 792-3873
Fax: (713) 745-2999
E-mail: rpasqual@mdanderson.org

WEBSITE

http://www.mdanderson.org/labs/pasqualini

EDUCATION

Degree-Granting Education

Ludwig Institute for Cancer Research, São Paulo Branch, Institute of Chemistry, University of São Paulo, 1990, Ph.D. in Biochemistry

Postgraduate Training

Postdoctoral Research Fellow, Cell Biology, Harvard Medical School, Joint Program in Neonatology, Boston, MA, Dr. Merton Bernfield, 1990-1991

Postdoctoral Research Fellow, Cellular and Molecular Biology, Harvard Medical School, Dana Farber Cancer Institute, Tumor Virology Division, Boston, MA, Dr. Martin Hemler, 1991-1994

Senior Research Fellow, Cellular and Molecular Biology, The Burnham Institute, La Jolla, CA, Dr. Erkki Ruoslahti, 1994-1996

CREDENTIALS

Board Certification

Licensure(s)
Active: N/A
Inactive: N/A

EXPERIENCE/ SERVICE

Academic Appointments

Associate Professor, Department of Genitourinary Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX, 1999-2003

Assistant Professor, The Burnham Institute, La Jolla, CA, 1997-1999

Academic Administrative Appointments/Responsibilities

N/A

Institutional Committee Activities

Member, The University of Texas-Houston Graduate School of Biomedical Sciences: Virology and Gene Therapy Program Committee, 2001 – Present

Member, Committee for the Review of The University of Texas M. D. Anderson Cancer Center Prostate Cancer SPORE Pilot Projects, 2001 – Present

Member, The University of Texas M. D. Anderson Cancer Center Prostate Cancer SPORE Executive Committee, 2001 – Present

Member, The University of Texas - Study Section Review Committee for Basic Research Projects, 2001 – 2003

Member, The University of Texas Postdoctoral Advisory Committee, 2001 – 2004

Member, The University of Texas M. D. Anderson Cancer Center Prostate Cancer SPORE External Advisory Committee, 2002 – Present

Member, Tissue Acquisition Committee, 2003 – Present

Member, Promotion and Tenure Committee, 2003 – 2006

Member, DVMS Core Grant Program Income Advisory Committee, 2003 – Present

Member, Education Subcommittee, 2004

Member, Experimental Therapeutics Search Committee, 2004 - 2005

Member, Extramural Programs Committee, 2004-present

Member, Basic Sciences Research Symposium Planning Committee Meeting, 2004 – 2005

Member, DNA Sequencing Facility Managing Committee, 2004 – Present

Member, Internal Advisory Board Revised Report for the Department of Experimental Diagnostic Imaging, 2006-present

Member, Advisory Steering Committee, Department of Extramural Programs, The United States-Middle East Partnership for Breast Cancer Awareness Research, The University of Texas M. D. Anderson Cancer Center, 2007 – present

Member and Chair, Advisory Steering Committee, Department of Extramural Programs, The United States-South America – Susan G. Komen Partnership for Breast Cancer Awareness Research, The University of Texas M. D. Anderson Cancer Center, 2007 – present

Member, Annual Faculty Recognition and Awards Program Selection Committee, 2007

Other Appointments/Responsibilities

Media/Communications Activities

Television

NBC Nightly news, KPRC-TV, Ch. 2. A story on M. D. Anderson's resurgence and rapid rise in patient growth. This story, which came as a direct result of the recent page-one Wall-Street Journal story, 2000.

CNN shooting with Dr. Mendelsohn, 2001.

Worked with PBS "NOVA" on an hour-long special on anti-angiogenesis and other new therapies, 2001.

7 News Boston, Diet Discovery, http://www1.whdh.com/features/articles/healthcast/DBM203/, 05/10/04.

ABC 13 Eyewitness News, Cancer research uncovers shot that could melt fat away, http://abclocal.go.com/ktrk/health/051004_health_fatcells.html, 05/10/04.

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News 10, Fat Cells, http://www.news10.com/Global/story.asp?S=1862161, 05/12/04.

Print

National

New York Times, Patent Report, Method of Identifying molecules that home to a selected organ in vivo, 1996.

Business Week, Protein tags chase down tumors, 1996.

San Diego Tribune, Researchers report gains in molecular tag study, 1996.

S. Komen Press Release, The Susan G. Komen Breast Cancer Foundation Announces 1998 Postdoctoral Fellowship Grantees, 1998.

Appeared on the cover of M. D. Anderson's "Research Milestones" brochure, 2001.

Urology Times, Molecular 'map' will allow study of tissue markers: urothelial address mapping will enable targeted imagining, diagnosis, and therapy, 2001.

The New York Times Company, Science Times, How cells know where to exit the bloodstream to go to work, 2002.

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ScienceNow, A new angle on targeted drug design, http://sciencenow.sciencemag.org/cgi/content/full/2002/204/1, 02/04/02.

Texas Medical Center News, Researchers identify "zip codes" in the human blood vessel system, http://www.tmc.edu/tmcnews/02_15_02/page_15.html, 02/15/02.

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U.S. News & World Report, Cancer's fearsome travelers, http://www.usnews.com/usnews/health/articles/040405/5cancer.b.htm, 04/05/04.

Chemical & Engineering News, Chemical Highlights 2004, http://pubs.acs.org/cen/coverstory/8251/8251chemistry.html, 12/20/04.

The Boston Globe, Molecule found to trim fat in mice, http://www.boston.com/yourlife/health/diseases/articles/2004/05/10/molecule_found_to_trim_fat_in_mice/, 05/10/04.

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The Christian Post, Early stage anti-obesity drug shows promise, http://www.christianpost.com/article/technology/91/early.stage.anti-obesity.drug.shows.promise/1.htm, 05/12/04.

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ISTOÉ, Ciencia, O vírus de ouro, 2006.

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Slashdot, Molecule cuts off fat's food supply, http://science.slashdot.org/article.pl?sid=04/05/11/0022203&threshold=4&mode=nested&commentsort=0, 05/10/04.

SurgeryNews.net, Blood feud kills off fat cells, http://www.surgerynews.net/news/0504/fat50404.htm, 05/10/04.

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WebMD Medical News, Obesity treatment makes fat vanish, http://webcenter.health.webmd.netscape.com/content/Article/86/99202.htm?z=1728_00000_1000_nb_01, 05/10/04.

Wired News, Blood feud kills off fat cells, http://www.wired.com/news/medtech/0%2C1286%2C63388%2C00.html?tw=wn_tophead_4, 05/10/04.

British Nursing News Online, Want to be slim? Cut your blood supply, http://www.bnn-online.co.uk/news_datesearch.asp?page=4&SearchDate=11/May/2004&Year=2004, 05/10/04.

Better Humans, Starving fat cells could treat obesity drug kills cells that feed fat tissue, http://www.betterhumans.com/News/news.aspx?articleID=2004-05-11-1, 05/11/04.

Biological Procedures Online, Obesity reversed in mice by destroying blood vessels that service fat cells, http://www.biologicalprocedures.com/bpo/general/news/040510/05.htm, 05/11/04.

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Benvenuti nel sito Le Scienze.it, Un bio-shuttle tutto d'oro *Lo "scafo" viene fatto costruire direttamente dal virus* http://www.extrapola.com/app/WebObjects/Content.woa/WebRawNews/2006/January/24/67145858/Benvenuti_nel_sito_Le_Sci.html, 01/24/06.

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UOL Vestibular, Banho de ouro cria vírus precioso, http://vestibular.uol.com.br/atualidades/ult1685u224.jhtm, 02/09/06.

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http://www.asbmb.org/asbmb/site.nsf, 2006

http://www.aacr.org/home/scientists/meetings--workshops/special-conferences/previous-special-conferences/innovations-in-prostate-cancer-research.aspx, 2006

Other

Hosted former President H. W.George Bush and Dr. Mendelsohn in the lab for video to be used for internal purposes as well as media distribution, 2001.

NPR, Science Friday, Morning Edition, Scientists optimistic over fat buster, http://www.npr.org/templates/story/story.php?storyId=1890304, 05/10/04.

The PNAS website receives almost 2 million hits every week and in 2004 over 10 million article pdfs were downloaded. This article was among the top 100 articles accessed. Pasqualini R and Arap W. Hybridoma-free generation of monoclonal antibodies. Proc Natl Acad Sci USA 101:257-9, 2004.

Host to Italian Consulate, Dr. Cristiano Maggipinto, 05/07

Odyssey Mini Symposium, Grand Rounds and Luncheon, The University of Texas M. D. Anderson Cancer Center, Houston, TX, 05/18/07

Host to Brazilian Consulate, Carlos Alberto de Azevedo Pimentel, General Consul, 06/07

Office of Development

Prostate Cancer Research Program, Office of Development Fund Raising, Indian Wells, CA, 2000.

Gillson-Longenbaugh Foundation Luncheon, 2001 – 2007.

New York Reception and Dinner, hosted by Michael Bartolotta, Board of Visitors Member, Harvard Club of New York, New York, NY, 11/03.

Participated with MDACC, Dr. Mendelsohn and the Office of Development, International Center, in the Turkish Minister of Health Site Visit, 04/04.

Host to Mr. Ross Perot, 05/04

Host to Mr. Marcus, 03/04

Dr. Robert Cohen/Marcus Foundation Site Visit, Houston, TX, 2004-2007.

Participated with MDACC, Dr. Mendelsohn and the Office of Development in the Bert Fields (Investment Banking and Oil) Site Visit, 01/23/05.

Participated with MDACC, Dr. Mendelsohn and the Office of Development in the David L. Van Andel (Amway Corporation/Van Andel Institute) Site Visit, 02/10/05.

Participated with MDACC, Office of Development in Bert Fields Site Visit, 01/31/06.

Participated with MDACC, Office of Public Affairs, 2004 – 2005

Conquest Annual Report, 01/06/06.

Participated with MDACC, Office of Development in Reagent Brian Haley Site Visit, 07/06.

Participated with MDACC, Dr. Mendelsohn in the Annual Aspen Seminar and Hines Reception, 07/06.

Invited speaker at the V Foundation Fund Raiser, Napa Valley, CA, 08/06.

Participated with MDACC, the Anderson Foundation fund-raising meeting, 05/07.

Consultantships

Zafgen, Inc., Scientific Advisory Board
Leonardo Biosystems, Inc., Scientific Advisory Board
MolMED, Scientific Advisor
Transmolecular Inc., Scientific Advisor
Becton, Dickinson and Company, Scientific Advisor

Military or Other Governmental Service

N/A

HONORS AND AWARDS

International Agency for Research on Cancer (IARC), Research Fellowship, 1990

Arthritis Foundation, Research Fellowship, 1993 – 1996

The Susan Komen Foundation, Research Fellowship, 1996 – 1997

AACR-Susan G. Komen Career Development Award in Basic Cancer Research, 1999

Angel Works Award, 2000

The Gillson-Longenbaugh Foundation Awards, 2000 - 2007

The V Foundation Award on Translational Cancer Research, 2001

Randall & Dewey Award, 2002

Golfers Against Cancer Foundation Award, 2003

Recipient of the Living Legend Faculty Achievement Award, 2005 – 2006

Marcus Foundation Award, 2006-2009

Recipient, Fellows of the M. D. Anderson Research Trust Award, 2006
(jointly with Wadih Arap, M.D., Ph.D.)

Among the top 400 inventors accounting for 99% of patents filed since 1946, 2007

RESEARCH

Grants and Contracts (funded and pending) – past 5 years
Funded (Principal Investigator)

Principal Investigator, NIH, R01CA78512, A receptor for tumor-homing peptides in angiogenic vasculature, 9/1/1999 – 06/30/04, $689,207 ($182,966/year).

Principal Investigator, NIH, 1R01CA88106, Targeted delivery of genes to angiogenic vasculature, 1/10/00 – 11/30/05, $570,769 ($121,001/year).

Principal Investigator, The Gillson-Longenbaugh Foundation Award, Cancer treatment by targeted drug therapy, 08/01/00 – 07/31/07, $728,000 ($105,000/year).

Principal Investigator, The V Foundation Award, Award Number 11-99-09932, Translation of the molecular diversity of blood vessels into vascular targeting applications, 1/1/01 – 12/31/04, $600,000 ($200,000/year).

Principal Investigator, NIH, P50CA90270 (PP-2), The University of Texas M. D. Anderson SPORE in prostate cancer, (Project 2 - Targeting prostate cancer bone metastasis), Program Director: Christopher Logothetis, M.D., 06/1/01 – 12/31/06, $597,267 ($168,635/year).

Principal Investigator, NIH, U01CA91134, Contrasting properties of integrin cytoplasmic domains, 9/4/01 – 8/31/06, $578,120 ($151,000/year)

Principal Investigator, U.S. Department of Defense, Innovator Award Grant: DAMD17-03-1-0384, Sub award Agreement No. R14101-7200003, Seamless integration of detection and therapy for breast cancer using targeted engineered nanoparticles, Program Director: Dr. Naomi Hallas, Rice University, 05/15/03 – 05/14/08, $420,000 (Sub-award $133,333/year)

Principal Investigator, U.S. Department of Defense, BC030054, Selection of therapeutic & diagnostic targets by phage display technology, center of excellence, Program Director: Saraswaiti Sukumar, Ph.D., 07/01/04 – 06/30/09, $964,623 (Sub award $185,431/year).

Principal Investigator, NIH, R01CA103830, Optical systems for in vivo molecular imaging of cancer, Program Director: Rebecca Richards Kortum, 08/01/04 – 07/31/09, $2,164,607 (Sub award $95,533/year).

Principal Investigator, NIH, P50CA100632, The University of Texas M. D. Anderson SPORE in Leukemia, (Project 7 – Identification of therapeutic targets for leukemia by phage display profiling of leukemia cell lines and patient-derived samples), Program Director: Hagop M. Kantarjian, M.D. and Jean-Pierre Issa, M.D, 08/16/04 – 04/30/08, $170,000.

Principal Investigator, NIH, P50CA091846, Developmental research award from the developmental research. Director: Colin Dinney, M.D. The developmental research program of the genitourinary cancer SPORE, developmental research award - an artificial pore-forming protein with anti-tumor activity, 09/01/04 – 08/31/05, $30,000.

Principal Investigator, Prostate Cancer Foundation Award, Targeting the interleukin 11R in prostate cancer metastasis, 01/01/05 – 12/31/05, $100,000.

Principal Investigator, W8XWH-05-2-0027, U.S. Department of Defense, 04091003, IMPACT: Imaging and molecular markers for patients with lung cancer: approaches with molecular targets, complementary/innovative treatments, and therapeutic modalities, Program Director: Waun Ki Hong, M.D., 02/01/05 – 03/01/09, $467,111 ($110,066/year).

Principal Investigator, NIH, R01 HL081658, Regulatory roles for vascular peptidases in angiogenesis, 08/01/05 – 07/31/09, $1,250,000 ($250,000/year).

Principal Investigator, Marcus Foundation (Bast) (PP4): Molecular targeting using vascular zip codes, 07/10/06 – 07/09/09, $330,264 (current year direct cost).

Principal Investigator, NCI, CA122568, Ligand-directed tumor targeting in preclinical models. 04/01/07 – 03/31/12 $1,250,000 (250,000/year).

Principal Investigator, Prostate Cancer Foundation, Pre-clinical studies toward the translation of IL 11R targeting ligands in prostate cancer. 04/05/07-06/04/08 $100,000

Principal Investigator, Defense Advanced Research Projects Agency (DARPA), BAA-07-21, #P-5317-MS-DRP, Defense Sciences Research and Technology, "Vascular and Lymphatic Targeting." 07/31/07-07/30/08, $338,000 (one-year).

Funded (Co-Principal Investigator, full signature authority)
Co-Principal Investigator, NIH, P50CA83639, PP-DRP9, Pilot Project of The University of Texas M. D. Anderson Cancer Center SPORE in Ovarian Cancer Award, Identification of tumor markers in ovarian cancer. Director: Robert Bast, M.D, SPORE in Ovarian Cancer, 9/30/99 – 08/31/05, $50,000.

Co-Principal Investigator, Juvenile Diabetes Foundation, 1-2001-291, Targeting angiogenic vasculature in the retina, 01/01/01 – 02/28/05, $404,999.

Co-Principal Investigator, NIH, P50CA90270 (PP-4), University of Texas M. D. Anderson SPORE in Prostate Cancer, (Project 4 – Exploring the molecular diversity of blood vessels for diagnostic and therapeutic targeting in prostate cancer), Program Director: Christopher Logothetis, M.D., 06/01/01 – 12/31/06, $609,379 ($185,714/year).

Co-Principal Investigator, NIH, U54CA90810 (PP-3), Targeted assessment of antiangiogenic therapy, (Project 3: Implications of the molecular heterogeneity of tumor blood vessels), Program Director: James L. Abbruzzese, M.D., 07/16/01 – 12/31/06, $790,197 ($143,440/year).

Co-Principal Investigator, U.S. Department of Defense, DAMD17-02-1-0257, Probing surface heterogeneity of metastatic prostate cancer cells, 02/01/02 – 01/31/05, $541,383.

Co-Principal Investigator, NIH, R33CA103056, Stem cell-brain tumor interplay & in vivo phage display, 08/15/03 – 07/31/08 (no cost extension), $1,348,671 ($444,398/year).

Co-Principal Investigator, NIH, R01DK67683, Imaging tumor blood vessels in bone metastases from breast cancer, 09/10/03 – 08/31/08 (no cost extension), $1,159,105 ($231,821/year).

Co-Principal Investigator, NIH, R33CA103030, Mapping vascular diversity of human cancer, 03/15/04 – 02/28/08 (no cost extension), $843,376 ($279,099/year).

Co-Principal Investigator, NIH, R01 DK070770, Molecular diversity in bladder cancer, 05/01/05 – 04/30/10, $1,250,000 ($250,000/year).

Pending (Principal Investigator)
Principal Investigator, R33CA122668-01, Ligand-Directed Mapping of Molecular Targets in Cancer, 07/01/2007 - 06/30/2012, $1,250,000 ($250,000/year)

Principal Investigator, R21CA128466-01, Gold Nanoparticle-based Scaffolds for Targeted Imaging and Tissue Ablation, 04/01/2008 - 03/31/2011, $200,000 ($100,000/year)

Principal Investigator, NIH, R01CA127251, The interleukin 11 receptor in angiogenesis and tissue remodeling. 04/01/2008- 03/31/2013, $ 1,250,000 (250,000/year)

Principal Investigator, U.S. Department of Defense, Concept Award, "Molecular biopsy" for early diagnosis and disease monitoring in breast cancer patients, 07/01/07 – 06/30/08, $75,000.

Principal Investigator, NIH, Integration of vascular genomics and proteomics for diagnosis and therapy of cancer, 02/01/08 – 01/30/13, $1,479,193 ($487,847/year).

Principal Investigator, NIH, Prostate Cancer SPORE, Project 2, Targeting the IL11R in Prostate Cancer Bone Metastasis, 08/08/07-09/08/13

Principal Investigator, Susan G. Komen Foundation, Imaging vascular zip codes in a model of inflammatory breast cancer. 07/03/07-04/05/10, $350,000 over 3 years.

Principal Investigator, DOD PCRP IDEA Award, Ligand-directed and transcription profiling of prostate cancer. 07/08/08-09/08/11, $375,000 over 3 years.

Principal Investigator, Jeffrey Rosenzweig Foundation, Integration of genomics and proteomics for imaging and therapy in pancreatic cancer. 09/01/07-08/31/09, $50,000 (two-year).

Principal Investigator, NIH, 2007 NIH Director's New Innovator Award Program, "Seeing from Within: Novel Ligand Discovery for Human Atheroma Imaging," 09/28/07-03/31/12

Principal Investigator, NIH, 1R33CA16663-01A1, R33 on AAVP, Integration of vascular genomics and proteomics for diagnosis and therapy of cancer. 12/01/07-11/30/10, $1,465,324 Direct

Principal Investigator, NIH, 1R01CA133026-01, GDD IL 11 R0-1, Targeting the interleukin-11 receptor alpha in prostate cancer metastasis, 04/01/08-3/31/13, $1,250,000 Direct

Principal Investigator, NCI, R01CA133719-01, PA-07-165, Molecular Targeting of Lymphatic Endothelial Receptors for Ligand-directed Imaging, 04/01/08-03/31/2013, $1,250,000

Principal Investigator, NIH, 1R01CA133680-01, PA-070-070, Targeting Modulation of Angiogenesis by VEGFR Peptidomimetic Antagonists, 04/01/2008-03/31/2013, $785,032 Total Direct Cost

Principal Investigator, R01CA113864-01A2, Targeted Phage-Based Vectors for Systemic Delivery of Therapeutic Agents to Brain Tumors, 12/01/2008 - 11/30/2013, $1,250,000 ($250,000/year)

Pending (Co-Principal Investigator)
Co-Principal Investigator, 1R01EY017822-01, Targeted Phage-Based Nanoscaffolds for Imaging of Angiogenic Blood Vessels in Diabetic Retinopathy, 07/01/2006-06/30/2011, $1,250,000 ($250,000/year)

Co-Principal Investigator, 1R01CA125639-01, Ligand-directed and transcriptional profiling in kidney

cancer, 10/02/2008- 03/31/12, $1,250,000 ($250,000/year)

Co-Principal Investigator, 1R01CA134772-01, Assessing drug response in soft tissue sarcoma by targeted molecular-genetic imaging, 07/01/2008-06/30/2013, $1,250,000 ($250,000/year)

Co-Principal Investigator, 1R01CA134803-01, Cellular mechanisms mediating pro-metastatic activity of exogenous osteopontin, 07/01/2008 - 06/30/2012, $1,250,000 ($250,000/year)

Co-Principal Investigator, 1R01HL093659-01, Targeted Au-phage Scaffolds for Imaging in Cardiovascular Diseases, 07/01/2008-06/30/2013, $1,250,000 ($250,000/year)

Funded Protocols
LAB05-0154: Studies on Blood and Tumor Tissues from Patients with Lung Cancer

LAB05-0027: Laboratory Immunological Studies on Blood and Tumor Tissues from Patients with Genitourinary Cancers

LAB05-0257: Studies on Blood and Tumor Tissue from Patients with Breast Cancer

LAB05-0286: Immunological Studies on Blood and Tumor Tissues from Patients with Ovarian Cancer

LAB05-0459: Studies on Blood and Tumor Tissues from Patients with Brain Cancer

LAB04-0678: Pre-Clinical Development and Testing of New Therapeutic Agents for Chronic Lymphoid Malignancies

ACUF ID#: 11-04-10631, Targeting Therapies

ACUF ID#: 11-99-09932, Targeting Blood Vessels

ACUF ID#: 11-99-09933, Targeting Blood Vessels

ACUF ID#: 05-07-04981, Toxicological Studies of Targeted Peptides in Monkeys

Patents Granted and Pending (U.T. M. D. Anderson Cancer Center)

Tab No.	F&J/MDA/BS File Code	Filing/ Priority Date	Serial No.	Title	Status
1	UTSC:674USP1 MDA00-025	09/08/00	60/231,266	"Compositions and Methods for Organ and Tissue Targeting in Humans"	Converted
2.	UTSC:674US MDA00-025	01/17/01 09/08/00	09/765,101		Converted to provisional application
3	UTSC:850US MDA00-025B P001	03/07/03 09/08/00	10/363,203	"Adenoviral Targeting and Manipulation of Immune System Response Using Targeting Peptides"	Pending
4	UTFC:850WO* MDA00-025B P001PCT	09/07/01 09/08/00	PCT/US/01 28045		Nationalized
5	UTFC:850AU MDA00-025B P001AU	09/07/01 09/08/00	2001/ 290663		Pending
6	UTFC:850CA MDA00-025B P001CA	09/07/01 09/08/00	2,421,200		Pending
7.	UTFC:850EP MDA00-025B P001EP	09/07/01 09/08/00	01970682.9		Pending
8.	UTFC:850JP MDA00-025B P001JP	09/07/01 09/08/00	2002/ 525731		Pending
9.	UTSC:851US MDA00-025C P002	03/07/03 09/08/00	10/363,202	"Compositions and Methods for Targeting Peptides in Humans In Vivo"	Pending
10.	UTFC:851WO* MDA00-025C P002PCT	09/07/01 09/08/00	PCT/US01/2 8044		Nationalized
11.	UTFC:851AU MDA00-025C P002AU	09/07/01 09/08/00	2001/ 290662		Pending
12.	UTFC:851CA MDA00-025C P002CA	09/07/01 09/08/00	2,421,195		Pending

* Note that each of UTFC:850WO, UTFC:851WO, UTFC:852WO and UTFC:853WO base priority on 60/231,266, filed 9/08/00 (formerly our file ref. UTSC:674, now UTSC:850USP1)

Tab No.	F&J/MDA/BS File Code	Filing/ Priority Date	Serial No.	Title	Status
13.	UTFC:851EP MDA00-025C P002EP	09/07/01 09/08/00	01970681.1		Pending
14.	UTFC:851JP MDA00-025C P002JP	09/07/01 09/08/00	2002/ 525730		Pending
15.	UTSC:852US MDA00-025D P003	03/07/03 09/08/00	10/363,204	"Human and Mouse Targeting Peptides Identified by Phage Display"	Pending
16.	UTFC:852WO* MDA00-025D P003PCT	09/07/01 09/08/00	PCT/US01/2 7692		Nationalized
17.	UTFC:852AU MDA00-025D P003AU	09/07/01 09/08/00	2001/ 288843		Pending
18.	UTFC:852CA MDA00-025D P003CA	09/07/01 09/08/00	2,421,271		Pending
19.	UTFC:852EP MDA00-025D P003EP	09/07/01 09/08/00	01968603.9		Pending
20.	UTFC:852JP MDA00-025D P003JP	09/07/01 09/08/00	2002/ 525776		Pending
21.	UTSC:853US MDA00-025E P004	03/07/03 09/08/00	10/363,205	"Biopanning and Rapid Analysis of Selective Interactive Ligands"	Pending
22.	UTFC:853WO* MDA00-025E P004PCT	09/07/01 09/08/00	PCT/US01/2 8124		Nationalized
23.	UTFC:853AU MDA00-025E P004AU	09/07/01 09/08/00	2001/ 288914		Pending
24.	UTFC:853CA MDA00-025E P004CA	09/07/01 09/08/00	2,421,380		Pending

Tab No.	F&J/MDA/BS File Code	Filing/ Priority Date	Serial No.	Title	Status
25.	UTFC:853EP MDA00-025E P004EP	09/07/01 09/08/00	01968683.1		Pending
26.	UTFC:853JP MDA00-025E P004JP	09/07/01 09/08/00	2002/ 525828		Pending
27.	UTFC:854WO MDA00-025F P005PCT	09/07/01 09/08/00	PCT/US01/2 7702		Nationalized
28	UTSC:854US MDA00-025F P005	09/02/03 09/08/00	10/363,208	"Methods and Compositions for In Vitro Targeting"	Pending
29.	UTFC:854AU MDA00-025F P005AU	09/07/01 09/08/00	2001/ 290,652		Pending
30.	UTFC:854CA MDA00-025F P005CA	09/07/01 09/08/00	2,421,191		Pending
31.	UTFC:854EP MDA00-025F P005EP	09/07/01 09/08/00	01970671.2		Pending
32.	UTFC:854JP MDA00-025F P005JP	09/07/01 09/08/00	2002/ 525729		Pending
33.	UTSC:855USP1 MDA01-093 P008Z	07/18/01	60/306,506	"Anti-Angiogenic State in Mice and Humans with Retinal Photoreceptor Cell Degeneration"	Pending
34.	UTFC:855WO MDA01-093 P008PCT	07/17/02 07/18/01	PCT/US02/2 2971		Nationalized
35.	UTSC:855US MDA01-093 NA	01/20/04 07/18/01	10/484,550		Pending
36.	UTFC:855EP MDA01-093	07/17/02 07/18/01	02761131.8		Pending

Tab No.	F&J/MDA/BS File Code	Filing/ Priority Date	Serial No.	Title	Status
37.	UTFC:855CA MDA01-093	07/17/02 07/18/01	2,454,357		Pending
38.	UTFC:856WO MDA00-025CIP1 P009PCT	08/30/02 09/07/01	PCT/US02/27836	"Compositions and Methods of Use of Targeting Peptides Against Placenta and Adipose Tissues"	Nationalized
39.	UTSC:856US MDA00-025CIP1	03/08/04 09/07/01	10/489,071		Pending
40.	UTSC:856EP MDA00-025CIP1	04/06/04 09/07/01	Not received yet		Pending
41.	UTFC:856CA MDA00-025CIP1	08/30/02 09/07/01	Not received yet		Pending
42.	UTFC:857WO MDA04-113 P010PCT	10/30/02 08/30/02	PCT/US02/34987	"Compositions and Methods of Use of Targeting Peptides for Diagnosis and Therapy of Human Cancer"	Pending
43.	UTSC:858USP1 MDA03-071P1 P011Z	04/14/03	60/462,631	"Methods for Hybridoma-Free Production of Murine and Human Monoclonal Antibodies"	Pending
44.	UTSC:860USP1 MDA03-071P2 P013Z	11/24/03	60/524,701	"Methods for Ex Vivo Hybridoma-Free Production of Murine and Human Polyclonal and Monoclonal Antibodies and Generation of Immortalized Cell Populations"	Pending

Tab No.	F&J/MDA/BS File Code	Filing/ Priority Date	Serial No.	Title	Status
45.	UTSC:858US MDA03-071	04/14/04 04/14/03 & 11/24/03	10/824,627	"Methods for Ex Vivo Hybridoma-Free Production of Polyclonal and Monoclonal Antibodies and Generation of Immortalized Cell Populations"	Pending
46.	UTFC:858WO MDA03-071	04/14/04 04/14/03	PCT/US04/11427		Pending
47.	UTSC:859USP1 MDA03-125 P012Z	09/12/03	60/502,509	"Biopanning as an Approach to Study the Pathogenesis of an Invent Novel Treatment Modalities for Invasive Aspergillosis"	Pending
48.	UTSC:861USP1 MDA04-030 P014Z	12/31/03	60/533,650	"Compositions and Methods of Use of Targeting Peptides for Diagnosis and Therapy" (IL-11 Receptor Targeting)	Pending
49.	UTSC:872US MDA00-025CIP2	02/23/04 09/08/00	10/784,537	"Aminopeptidase A (APA) Targeting Peptides for the Treatment of Cancer"	Pending
50.	UTSC:890USP1 MDA04-089	07/10/04	60/586,814	"Composition and Methods Related to Peptides that Selectively Bind Leukemic Cells."	Pending
51.	UTSC:889 MDA04-083	11/16/2004	60/628,472	"Methods and Compositions Related to Phage Nanoparticles"	Provisional application filed
52.	UTXC:891 MDA04-093	11/16/2004	60/628,495	"Synchronous Selection of Homing Peptides for Multiple Tissues by in Vivo Phage Display"	Provisional application filed
53.	UTSC:916USP1	03/09/06		"Compositions and Methods Related to Profiling A Plurality of Cell Lines Based on Peptide Binding"	Provisional application filed
54.		04/01/06		"Targeted Manipulation of Gene Expression"	Pending
55.	UTSC:861USP1	08/07/07		"GRP78 Targeting Peptides and Methods of Employing the Same"	Pending

56.	UTSC:985USP1	11/25/07	"Tumor-homing peptides for targeted therapy and imaging"	Provisional application filed
57.	UTSC:991USP1	08/08/07	"VEGF-1/NRP-1 Targeting Peptides"	Provisional application filed

Patents Granted and Pending (The Burnham Institute)

Family	#	Patent/Patent Application No.	C&F Docket#	Patent Type
1		Method of Identifying molecules that home to a selected organ in vivo		
	1	5622699	P-LJ 1779	Parent
	2	08/813,273	P-LJ 2410	CIP of 08/526,710
	3	6068829	P-LJ 2621	CIP of 08/526,710
	4	09/226,985	P-LJ 3423	Cont. of 08/526,710
	5	09/227,906	P-LJ 3424	Cont. of 08/526,710
	6	96250195.3	FP-LJ 2173	Foreign
	7	96930824.6	FP-LJ 3014	Foreign
	8	US96/14600	FP-LJ 2174	Foreign
	9	2204535	FP-LJ 2533	Foreign
	10	9-512087	FP-LJ 2534	Foreign
	11	63740/98	FP-LJ 3062	Foreign
	12	99250432.4	FP-LJ 3859	Foreign
2		Molecules that home to a selected organ in vivo		
	13	09/228,866	P-LJ 3430	Cont. of 08/526,708
3		Molecules that home to various selected organs or tissues		
	14	09/042,107	P-LJ 2892	Parent
	15	09/722,250	P-LJ 4514	Cont. of 09/042,107
4		Tumor homing molecules, conjugates derived therefrom, and methods of using same		
	16	08/926,914	P-LJ 2725	Substitute of 08/710,067
	17	97942422.3	FP-LJ 3466	Foreign
	18	US97/16086	FP-LJ 2803	Foreign
	19	44122/97	FP-LJ 3463	Foreign
	20	2,265,484	FP-LJ 3464	Foreign
	21	10-513856	FP-LJ 3465	Foreign
5		NGR receptor and methods of identifying tumor homing molecules that home to angiogenic vasculature using same		
	22	09/139,802	P-LJ 3203	CIP of 08/926,914
		09/659,786	P-LJ 4296	Cont. of 09/139,802
	23	US98/18895	FP-LJ 3296	Foreign
	24	98948140.3	FP-LJ 4024	Foreign
	25	2000-511062	FP-LJ 4025	Foreign
6		Methods of identifying lung homing molecules using membrane dipeptidase		
	26	09/258,754	P-LJ 3443	CIP of 09/042,107
	27	US99/05284	FP-LJ 3406	PCT

Family	#	Patent/Patent Application No.	C&F Docket#	Patent Type
7		NG2 proteoglycan-binding peptides home to tumor vasculature		
	28	09/250,700	P-LJ 3433	Parent
	29	US00/01602	FP-LJ 3866	PCT
8		Homing pro-apoptotic conjugates and methods of using same		
	38	09/235,902	P-LJ 3371	Parent
	39	US00/01602	FP-LJ 3866	PCT
9		Chimeric prostate-homing peptides with-proapoptotic activity		
	40	09/489,582	P-LJ 3844	Parent
	41	09/765,086	P-LJ 4575	
10	42	Methods of targeting angiogenic vasculature using gelatinase inhibitors		
		09/552,805	P-LJ 3802	

Grant Reviewer/Service on NIH/Other Study Sections

NIH Study Section, RFA, Gene Transfer Principles for Heart, Lung, and Blood Disorders, 1997

Department of Defense Study Section, Prostate Cancer Research Program, 1998, 1999

NIH Study Section, NCI, SBIR/STTR Program, 1999

NIH Study Section, NCI, Developmental Therapeutics Program, RAID, 1999

NIH Study Section, NCI, Angiogenesis PAR, 1999

NIH Study Section, NCI, Molecular Target Drug Discovery RFA, 2000

Dutch Cancer Society, 2000 - Present

Italian Cancer Research Foundation, 2001 - Present

The WELLCOME Trust, London UK, 2001 - Present

NIH Study Section, NCI, P0-1 Site Visit Panel, Harvard Medical School, 2001

Department of Energy, Targeted Therapeutics Research Program, 2001

NIH Study Section, NCI, Molecular Target Drug Discovery RFA, 2001

NIH Study Section, NIDDK, P0-1 Site Visit Panel, UT Southwestern, 2001

NCI, Parent Committee C, 2002

NCI, P0-1 Site Visit Panel, Scripps Research Institute, CA, 2002

AACR Special Meetings Committee, 2002

NCI, P0-1 Site Visit Panel, Ralph H. Johnson VA Hospital, South Carolina, 2002

NCI, Path A Study Section, 2002

The Canadian Institutes of Health Research, CIHR, 2002

Extramural Advisory Board, Vascular Biology Faculty of the Center for Cancer Research, NCI, 2002

Italian Ministry of Education, 2002 - Present

NIDA, CEBRA Stage I Review, 2002

Trainee Recognition Day, 2003

U.S. Department of Defense, BCRP Programmatic Review Meeting, Committee, 2003-2004

NCI, Intramural Review Panel, 2003

NCI, Genitourinary Cancer SPORE Review, 2003

NCI, P0-1 site visit, Ralph H. Johnson VA Hospital, South Carolina, 2003

National Cancer Institute Mouse Cancer Genetics Program Site Visit, Frederick, Maryland, 2003

AACR Grants Committee (Gertrude Elion Award, Career Development Awards, Research Fellowship Awards), 2004

NIH Road Map Initiative, R0-3 Study Section, 2004

Singapore Cancer Research Foundation, 2004

NCI, Head & Neck SPORE Review, 2004

NIH, Special Emphasis Panel (SEP): High Throughput Molecular Screening Assay Development", 2004.

U.S. Department of Defense, Breast Cancer Research Program (BCRP) Programmatic Review, 2004.

NCI, Vascular Biology Study Section, 2004-Present

NCI, SBIR Study Section, 2005

NCI, LRP Study Section, 2005

The Israel Science Foundation, Israel Academy of Sciences and Humanities, 2005

NCI, Centers of Cancer Nanotechnology Excellence, 2005

Special Emphasis Panel for the Review of Applications Submitted in Response to RFA-05-024, entitled "Centers of Cancer Nanotechnology Excellence" (CCNEs), 2005

Special Emphasis Panel, In Vivo Cellular and Molecular Imaging Centers (ICMICS), 2005

MRC Molecular and Cellular Medicine Board, UK, 2005

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Nanotechnology Panel, Association of Health Care Journalists Conference, 2006

The BSF (United States-Israel Binational Science Foundation), 2006

LRP Review, 2006

Special Emphasis Panel/Scientific Review Group 2007/01 ZRG1 BST-R (50) R Meeting, 2006

Ad Hoc Member, Breast Cancer SPORE, Pilot Projects Review Panel. Principal Investigator: Dr. Carlos Arteaga, Vanderbilt University School of Medicine, 2006

Ad Hoc Member, NIH Study Section, Bioengineering Research Partnership: Bioengineering Sciences and Technologies IRG (BST), 2006

Member, Scientific Program Committee Subgroup of Society for Molecular Imaging, 2007

Ad Hoc Member, Gene and Drug Delivery Study Section, NIH/NCI, 2007

Member, Israel Science Foundation, 2007

Member, IMPACT BCRP, DOD, 2007

Member, Cancer Research UK, 2007

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a. Articles in Peer-Reviewed Journals

1. Brentani RR, Ribeiro SF, Potocnjak P, **Pasqualini R**, Lopes JD, Nakaie CR. Characterization of the cellular receptor for fibronectin through a hydropathic complementarity approach. Proc Natl Acad Sci U S A 85:364-7,1988.

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65. Kolonin MG, Sun J, Do KA, Vidal CI, Ji Y, Baggerly KA, **Pasqualini R,** Arap W. Synchronous selection of homing peptides for multiple tissues by in vivo phage display. FASEB J 20:979-81, 2006.

66. Souza GR, Levin CS, Hajitou A, **Pasqualini R**, Arap W, Miller JH. In vivo detection of gold-imidazole self-assembly complexes: NIR-SERS signal reporters. Anal Chem 78:6232-37, 2006.

67. Tuder RM, Yoshida T, Arap W, **Pasqualini R**, Petrache I. State of the art. Cellular and molecular mechanisms of alveolar destruction in emphysema: An evolutionary perspective. Proc Am Thorac Soc 3:503-10, 2006.

68. Kzhyshkowska J, Workman G, Cardó-Vila M, Arap W, **Pasqualini R**, Gratchev A, Krusell L, Goerdt S, Sage EH. Novel function of alternatively activated macrophages: stabilin-1 mediated clearance of SPARC. J Immunol 176:5825-32, 2006.

69. Hajitou A, Rangel R, Trepel M, Soghomonyan S, Gelovani JG, Alauddin MM, **Pasqualini R**, Arap W. Design and construction of targeted AAVP vectors for mammalian cell transduction. Nat Protoc 3:523-31, 2007.

70. Rangel R, Sun Y, Guzman-Rojas L, Ozawa MG, Sun J, Giordano RJ, Van Pelt CS, Tinkey PT, Behringer RR, Sidman RL, Arap W, **Pasqualini R**. Impaired angiogenesis in aminopeptidase N-null mice. Proc Natl Acad Sci U S A 104:4588-93, 2007.

71. Ji Y, Yin GS, Tsui KW, Kolonin MG, Sun J, Arap W, **Pasqualini R**, Do KA. Bayesian mixture models for complex high dimensional count data in phage display experiments. Appl Statist 56:1-14, 2007.

72. Soghomonyan S, Hajitou A, Rangel R, **Pasqualini R**, Arap W, Gelovani JG, Alauddin M. Molecular PET imaging of HSV1-tk reporter gene expression using ^{18}F-FEAU. Nat Protoc 2:416-23, 2007.

73. Lahdenranta J, Sidman RL, **Pasqualini R**, Arap W. Treatment of hypoxia-induced retinopathy with targeted proapoptotic peptidomimetic in a mouse model of disease. FASEB J 12:3272-8, 2007.

74. Bover LC, Cardó-Vila M, Sun J, Aggarwal B, Arap W, **Pasqualini R**. A previously unrecognized protein-protein interaction between TWEAK and CD163: potential biological implications. J Immunol 178:8183-94, 2007.

75. Jaalouk DE, Schlingemann RO, Ozawa MG, Sun J, Lahdenranta J, **Pasqualini R**, Arap W. The original PAL-E monoclonal antibody recognizes a VEGF-binding site within neuropilin-1. Cancer Res 67:9623-9, 2007.

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b. Invited Articles

1. **Pasqualini R**, Ruoslahti E. Searching for a molecular address in the brain. Mol Psychiatry 1:421-2, 1996.

2. **Pasqualini R**, Ruoslahti E. Tissue targeting with phage peptide libraries. Mol Psychiatry 1:423, 1996.

3. Arap W, **Pasqualini R**, Ruoslahti E. Chemotherapy targeted to tumor vasculature. Curr Opin Oncol 10:560-5, 1998.

4. Koivunen E, Arap W, Rajotte D, Lahdenranta J, **Pasqualini R**. Identification of receptor ligands with phage display peptide libraries. J Nucl Med 40:883-8, 1999.

5. **Pasqualini R**. Vascular targeting with phage peptide libraries. Q J Nucl Med 43:159-62, 1999.

6. Arap W, **Pasqualini R**. The human vascular mapping project. Selection and utilization of molecules for tumor endothelial targeting. Haemostasis 1(Suppl 31): 30-31, 2001.

7. Kolonin M, **Pasqualini R**, Arap W. Molecular addresses in blood vessels as targets for therapy. Curr Opin Chem Biol 5:308-13, 2001.

8. **Pasqualini R**, Arap W, McDonald DM. Probing the structural and molecular diversity of tumor vasculature. Trends Mol Med 8:563-71, 2002.

9. Trepel M, Arap W, **Pasqualini R**. In vivo phage display and vascular heterogeneity: implications for targeted medicine. Curr Opin Chem Biol 6:399-404, 2002.

10. **Pasqualini R**, Arap W. Translation of vascular diversity into targeted therapeutics. Ann Hematol 81(Suppl 2):S66-7, 2002.

11. Arap W, **Pasqualini R**. In vivo screening: Vascular Maps. Nature Reviews Drug Discovery 1:174, 2002.

12. **Pasqualini R**, Barbas CF 3rd, Arap W. Putting a Finger on Angiogenesis. Nat Med 2002.

13. Sokolov K, Aaron J, Hsu B, Nida D, Gillenwater A, Follen M, MacAulay C, Adler-Storthz K, Korgel B, Descour M, **Pasqualini R**, Arap W, Lam W, Richards-Kortum R. Optical systems for in vivo molecular imaging of cancer. Technol Cancer Res Treat 2:491-504, 2003.

14. Zurita AJ, Arap W, **Pasqualini R**. Mapping tumor vascular diversity by screening phage display libraries. J Control Release 91:183-6, 2003.

15. Hajitou A, **Pasqualini R**, Arap W. Vascular targeting: Recent advances and therapeutic perspectives. Trends Cardiovasc Med 16:80-8, 2006.

16. Sergeeva A, Kolonin MG, Molldrem JJ, **Pasqualini R,** Arap W. Display technologies: application for the discovery of drug and gene delivery agents. Adv Drug Deliv Rev 58:1622-54, 2006.

17. Sato M, **Pasqualini R**, Arap W. Molecular Targets on Blood Vessels for Cancer Therapies Currently in Clinical Trials. Oncology, 11:1346-1353, 2007.

18. **Pasqualini R,** Arap W. Therapeutic applications of vascular proteomics. Nat Rev Drug Disc (In press).

19. Arap W, Trepel M, Zetter BR, **Pasqualini R**. Meeting Report: Innovations in Prostate Cancer Research (In press).

c. Editorials & Letters

1. Trepel M, Arap W, **Pasqualini R**. Exploring vascular heterogeneity for gene therapy targeting. Gene Ther 7:2059-60, 2000.

2. Cardó-Vila M, Arden KC, Cavenee WK, **Pasqualini R**, Arap W. Is annexin 7 a tumor suppressor gene in prostate cancer? Pharmacogenomics J 1:92-4, 2001.

3. **Pasqualini R**, McDonald DM, Arap W. Vascular targeting and antigen presentation. Nat Immunol 2:567-8, 2001.

4. **Pasqualini R**, Barbas CF III, Arap W. Vessel maneuvers: zinc fingers promote angiogenesis. Nat Med 8:1353-4, 2002.

5. Kontoyiannis DP, **Pasqualini R**, Arap W. Aminopeptidase N inhibitors and SARS. Lancet 361:1558, 2003.

6. Arap W, **Pasqualini R**. Engineered embryonic endothelial progenitor cells as therapeutic Trojan horses. Cancer Cell 5:406-8, 2004.

d. Other Articles

N/A

e. Abstracts (since relocation to The University of Texas M. D. Anderson Cancer Center)

1. Arap W, **Pasqualini R**. The human vascular mapping project. CaP CURE 8th Annual Scientific Retreat, Lake Tahoe, NV, September 6-9, 2001.

2. Giordano R, Cardó-Vila M, Lahdenranta J, **Pasqualini R**, Arap W. Biopanning and rapid analysis of selective interactive ligands #1141. Proc Am Assoc Cancer Res 42:213, 2001.

3. Kolonin MG, Arap W, **Pasqualini R**. Targeting Physiological and Pathological Blood Vessel Formation with in Vivo Phage Display #4416. Proc Am Assoc Cancer Res 42:822, 2001.

4. Oh YW, Mohiuddin I, Hong WK, Putnam J, Arap W, **Pasqualini R**. Bronchial and pulmonary artery perfusion of lung metastases #2193. Proc Am Assoc Cancer Res 42:407, 2001.

5. Arap W, Kolonin M, Trepel M, Baggerly K, Lahdenranta J, Giordano RJ, Cardó-Vila M, Yao V, Mintz PJ, Ardelt PU, Flamm A, Valtanen H, Weavind LM, Hicks M, Troncoso P, Pollock RE, Botz GH, Bucana C, Koivunen E, Cahill D, Pentz RD, Do KH, Logothetis CJ, **Pasqualini R.** Towards mapping the human vasculature by in vivo phage display #2928. Proc Am Assoc Cancer Res 43:591, 2002.

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7. Cardó-Vila M, Arap W, **Pasqualini R.** Contrasting properties of integrin cytoplasmic domains revealed by phage display #4181. Proc Am Assoc Cancer Res 43:844, 2002.

8. Cardó-Vila M, Arap W, **Pasqualini R.** Isolation of signaling molecule involved in angiogenesis mediated by beta 5 integrin cytoplasmic domain. Department of Army, Era of Hope, September 2002.

9. Giordano RJ, Lahdenranta J, Shapiro L, Arap W, **Pasqualini R**. Mass spectroscopy analysis of metalloproteases from tumor blood vessels: Aminopeptidase-N (CD13) activity upon angiogenic factors as a model #918. Proc Am Assoc Cancer Res 43:183, 2002.

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11. Oh YW, Mohiuddin I, Liu T, Hong WK, Putnam J, McDonald D, Arap W, **Pasqualini R.** Visualization of microvasculature development in pulmonary metastases #4453. Proc Am Assoc Cancer Res 43:898, 2002.

12. Vidal CI, Broaddus RR, Lu KH, Ellis LM, Arap W, **Pasqualini R.** Identification of molecular markers in ovarian cancer #3013. Proc Am Assoc Cancer Res 43:608, 2002.

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14. Vidal CI, Mintz PJ, Broaddus R, Lu K, Ellis LM, Arap W, **Pasqualini R.** Identification of molecular markers in ovarian cancer. 10th Spore Investigator's Workshop, Chantilly, VA, July 13-16, 2002.

15. Bover LC, Holbeck S, Scudiero DA, Sausville E, **Pasqualini R**, Arap W. Targeting the NCI 60-cell panel by biopanning and rapid analysis of selective interactive ligands #4574. Proc Am Assoc Cancer Res 44:1049, 2003.

16. Bover LC, Holbeck S, Scudiero DA, Zurita AJ, Sausville E, **Pasqualini R**, Arap W. Targeting the NCI 60-Cell panel by biopanning and rapid analysis of selective interactive ligands. AACR-NCI-EORTC international conference: Molecular targets and cancer therapeutics, 2003.

17. Cardó-Vila M, Arap W, **Pasqualini R.** αVβ5 Integrin-dependent programmed cell death triggered by a peptide mimic of annexin V. Gordon Conference, 2003.

18. Chen L, Giordano RJ, Ardelt PU, **Pasqualini R**, Arap W. A bi-specific phage system for target discovery and delivery in cancer #4554. Proc Am Assoc Cancer Res 44:1044, 2003.

19. Ellerby HM, Lee S, Andrusiak R, Ellerby LM, Chen SF, Kiyota T, del Rio G, Sugihara G, Bredesen DE, Arap W, **Pasqualini R**. A de novo designed protein with anti-tumor effects #6449. Proc Am Assoc Cancer Res 44:1478, 2003.

20. Hajitou A, Marini F, Lilley C, Moya C, Restel B, Arap M, Arap W, **Pasqualini R.** A new generation of targeted phage-based vectors for systemic gene delivery. Gordon Conference, 2003.

21. Kolonin MG, **Pasqualini R**. Analysis of human vascular proteome with phage display. Applied Biosystems, ABRF Conference, 2003.

22. Kolonin MG, Saha PK, Chan L, **Pasqualini R**, Arap W. Therapeutic targets of prohibition in adipose tissue vasculature. ASCI/AAP Joint Meeting, 2003.

23. Lahdenranta J, Giordano RJ, Langley R, Fidler IJ, **Pasqualini R**, Arap W. Selection of phage libraries on lung endothelium-derived cells by BRASIL and in vivo validation of a homing-peptide to the pulmonary vasculature #2763. Proc Am Assoc Cancer Res 44:631, 2003.

24. Langley RR, Lahdenranta J, Giordano R, Ramirez K, Tsan R, Arap W, **Pasqualini R**, Fidler IJ. Phenotypic diversity of endothelial cells, #2957. Proc Am Assoc Cancer Res, 44:675, 2003.

25. Mintz P, Kim J, Do KA, Wang X, Zinner RG, Cristofanilli M, Arap MA, Hong WK, Troncoso P, Logothetis CJ, **Pasqualini R**, Arap W. Fingerprinting the circulating repertoire of antibodies from cancer patients #5629. Proc Am Assoc Cancer Res 44:1290, 2003.

26. Oh YW, Mohiuddin I, Sun C, Putnam JB Jr, Hong WK, Arap W, **Pasqualini R**, Vasculature diversity in experimental melanoma metastases #5850. Proc Am Assoc Cancer Res 44:1342, 2003.

27. Zurita AJ, Troncoso P, Logothetis CJ, **Pasqualini R**, Arap W. A ligand peptide with potential for targeting prostate cancer metastatic to bone marrow #1659. 11th Spore Investigators Workshop. Proc Am Assoc Cancer Res 44:376, 2003.

28. Jaalouk DE, Kuniyasu A, Marini F, Ranta RM, Giordano R, Bover L, Sun C, Koivunen E, Arap W, **Pasqualini R.** Identification of therapeutic targets for leukemia by phage display profiling of leukemia cell lines and clinical samples. 12th SPORE Investigators' Workshop, 2004.

29. Juliana B, Giordano RJ, Kellen F, Oshiro SE, **Pasqualini R**, Arap W, Jorge K, Guilherme L. Phage display study using heart-infiltrating t-cell population rich in CD4+ isolated from rheumatic heart disease patients. 2004.

30. **Pasqualini R.** Combinatorial Screenings in Patients: The interleukin-11 receptor α as a marker for targeted Induction of apoptosis in advanced prostate cancer. 12th SPORE Investigators' Workshop, 2004.

31. **Pasqualini R**, Arap W. Targeted nanotracers for in vivo imaging and tissue ablation. Alliance for Nanohealth Workshop, 2004.

32. Zurita AJ, Hajitou A, Cardó-Vila M, Troncoso P, Logothetis CJ, **Pasqualini R**, Arap W. Preclinical development of an interleukin-11 receptor-targeted pro-apoptotic peptide against advanced prostate cancer #3190. 40th Annual Meeting of The American Society Of Clinical Oncology. Proc ASCO 2004.

33. Alauddin MM, Hajitou A, Soghomonyan S, Ozawa MG, Balatoni J, **Pasqualini R**, Arap W, Gelovani J. Micro-pet imaging of HSV-tk gene delivery and expression with [18F]-feau in tumor-bearing mice. The International Society of Molecular Imaging, Copenhagen, Denmark, 2005.

34. Sharma P, Tang ND, Arap W, **Pasqualini R**. Combinatorial targeting of the NY-ESO-1 cancer testis antigen. Cancer Research Institute, 2005.

35. Petrache I, Arap W, **Pasqualini R**, Flotte T, Tuder RM. Diagnostic and therapeutic applications of bacteriophage associated virus technologies in pulmonary emphysema. Nanomedicine 2:294, 2006.

36. Takahashi S, Nishimura S, Kuniyasu A, Nakayama H, Jaalouk D, Arap W, **Pasqualini R**. Identification of leukemia targeting peptide ligand having cell penetrating activity. J British Studies, 2007.

37. Mikhaylova A, Hajitou A, Kato Y, Sukumar S, Artemov D, Penet MF, Arap W, **Pasqualini R**, Bhujwalla ZM. Imaging targeted delivery of liposomes to tumor vasculature, ISMRM 2008.

38. Marchio S, Soster M, Castorino F, Sun J, Monti M, Pucci P, Dovetta D, Botta M, Punzoni M, Arap W, **Pasqualini R**, Bussolino F. Targeting the extracellular signature of metastatic colon cancers. AACR 2008.

39. Nava-Parada MP, Leatherman JM, Asquith JM, Jaffee EM, Arap W, **Pasqualini R**, Emens LA. A glucose-regulated peptide 78 (GRP78)-binding motif fused to the proapoptotic sequence d(KLAKLAK)2 can induce tumor immunity. AACR 2008.

40. Pastorino F, Di Paolo D, Loi M, Marchiò S, Soster M, Longhi R, Corti A, Allen TM, Bussolino F, Arap W, **Pasqualini R**, Ponzoni M. Phage display technology for novel tumor- and vascular-targeted therapies against neuroblastoma. AACR 2008.

f. Book Chapters

1. Brentani RR, **Pasqualini R**. Purification of the fibronectin receptor using the hydropathy complementarity approach. In: Receptor Purification, The Humana Press Inc, 1:451-457, 1990.

2. Hemler M, Bodorova J, **Pasqualini R.** Adhesion structures: CD29. In: Leukocyte Typing V, A.S. 4/72, Oxford University Press, Oxford, 1994.

3. Hemler M, Weitzman J, **Pasqualini R**, Kawaguchi S, Kassner P, Berdichevsky F. Structure, biochemical properties, and biological functions of integrin cytoplasmic domains. In: The Integrin: The Biological Problems, CRC Press Inc, Boca Raton, FL, pp. 1-35, 1994.

4. Koivunen E, Restel BH, Rajotte D, Lahdenranta J, Hagedorn M, Arap W, **Pasqualini R**. Integrin-binding peptides derived from phage display libraries. In Howlett AR, eds. Meth Mol Biol, Humana Press, Inc., 129:3-17, 1999..

5. Schneller M, Arap W, **Pasqualini R**. Immunoblotting of integrins. Meth Mol Biol 129:63-78, 1999.

6. **Pasqualini R.** Arap W. Phage displayed peptides as tools to explore vascular heterogeneity. In: J Martinez, JA Fehrentz (eds.), Peptides, pp. 26-30: EPS/Edicion EDK, 2001.

7. **Pasqualini R**, Arap W. Translation of vascular proteomics into individualized therapeutics. In: Pharmacogenomics in Search for Individualized Therapies, Wiley-VCH Press, pp. 525-530, 2002.

8. **Pasqualini R**, Arap W. Vascular targeting. In: The Encyclopedia of Cancer, Ed. Joseph R. Bertino, Academic Press Inc., New Brunswick, NJ, Second edition, Vol 4, pp. 501-7, 2002.

9. **Pasqualini R**, Arap W. Profiling the molecular diversity of blood vessels. Cold Spring Harbor Symposia on Quantitative Biology, Vol 67, pp. 223-5, 2002.

10. Kolonin M, **Pasqualini R**, Arap W. Mapping human vascular heterogeneity by in vivo phage display. In: Genetics of Angiogenesis, Ed. James B. Hoying, BIOS Scientific Publishers Ltd, Oxford, Ch.11: pp.181-187, 2003.

11. Vidal C, Cardó-Vila M, Lahdenranta J, Arap W, **Pasqualini R.** Targeting blood vessels in vivo by using phage display libraries. In: Targeted Therapy for Cancer, K.N. Syringos and K.J. Harrington, eds. (New York, NY, Oxford University Press Inc.), pp 250-255, 2003.

12. Lahdenranta J, Arap W, and **Pasqualini R.** The use of proteomics to map phenotypic heterogeneity of the endothelium. In: Aird W.C., eds. Endothelial Cells In Health and Disease, Taylor & Francis, pp 105-119, 2005.

13. Christianson DR, Ozawa MG, **Pasqualini R**, Arap W. Techniques to decipher molecular diversity by phage display. In Cardiovascular Proteomics book in Methods in Molecular Biology, Humana Press. 357:385-406, 2006.

14. Trepel M, Arap W, **Pasqualini R**. Selection, isolation, and identification of targeting peptides for ligand-directed gene delivery. Gene Transfer, Delivery and Expression of DNA and RNA: A Laboratory Manual. In: Friedman and Rossi, eds, Cold Spring Harbor Laboratory Press, New York. Chapter 30, pp. 359-369, 2007.

15. Zurita AJ, Arap W, **Pasqualini R**. Molecular characterization of the endothelium: A phage display perspective the endothelium. In: A Comprehensive Reference, The Endothelial Cell as Input-Output Device/Output/Proteome, Ed. Aird WC., Marcel Dekker, Inc., Chapter 100, pp. 898-908, 2007.

16. Trepel M, Pasqualini R, Arap W. Molecular Addresses Revealed by in vivo Phage Display. Methods in Enzymology, (In press).

g. Books (edited and written)

1. **Pasqualini R**, Arap W (eds.), Protein Discovery Technologies, Principles, Methods and Applications, Marcel Dekker/CRC Press, New York. (In Press).

h. Letters to the Editor
N/A

i. Manuals, Teaching Aids, Other Publications
1. **Pasqualini R**, Arap W, Rajotte D, and Ruoslahti E. In vivo selection of phage-display libraries. In: C. F. Barbas, III, D. R. Burton, J. K. Scott, and G. J. Silverman (eds.), Phage Display: A Laboratory Manual, Chapter 22, pp. 1-24, New York: Cold Spring Harbor Laboratory Press, 2000.

j. Other
N/A

EDITORIAL AND REVIEW ACTIVITIES

Editor/Service on Editorial Board(s)

Editor, Microvascular Research
Editor, Frontiers in Bioscience
Editor, Cancer Biology and Therapy
Editor, Molecular Cancer Research
Editor, Protein Discovery, CRC Press
Editor, Advanced Drug Delivery Review
Editor, Angiogenesis
Associate Editor, Cancer Research

Journal Reviewer

Nature
Nature Medicine
Nature Biotechnology
Nature Methods
Science
PNAS
BLOOD
EMBO Journal
Cancer Cell
J Clin Invest
Chemistry & Biology
Eur J Biochem
Cancer Research
Cancer Biology and Therapy
Molecular Cancer Therapeutics
Gene Therapy
Human Gene Therapy
Molecular Therapy
The American Journal of Pathology
Microvascular Research
Pharmacogenomics Journal
International J of Cancer
Clinical Cancer Research
J Natl Cancer Institute
Trends in Mol Med
Acta Biochem and Biophys Syn
Br J Cancer
Int J Rad Oncol
Arteriosclerosis, Thrombosis, and Vascular Biology
BioTechniques
FEBS J
Molecular Psychiatry

PLoS ONE
Trends in Immunology

TEACHING

Within Current Institution

Formal Teaching

Courses Taught

Instructor, GS04 0153, Human Gene Therapy: Basic Science/Clinical Trial, 2000

Instructor, GS04 0212, Mechanisms Cancer Therapeutics, 2002

Instructor, The University of Texas Medical School, Vascular Biology Course, 2004, 2005

Instructor, The University of Texas Medical School, Gene Therapy Course, 2002, 2003

Training Programs

Member, Virology and Gene Therapy, 1999 - Present

Program Mentor, CCSG – Cancer Biology & Metastasis Program, 1999 – Present

Program Mentor, Gene Targeting and Therapy Program, 1999 – Present

Program Mentor, GU Program, 1999 – Present

Program Mentor, Pharmacoinformatics Training Grant, 2004 – Present

Member, Vascular Biology, 2004 – Present

Member, Howard Hughes Medical Institute Graduate Training Grant entitled "Translational Bioengineering for Cancer Diagnostics and Therapeutics", 2006 – 2009

Other Educational Programs (since relocation to the University of Texas M. D. Anderson Cancer Center)

Information Exchange, Department of Molecular Genetics, 2000

Board of Visitors Annual Meeting, 2000

Department of Bioimmunotherapy, Division of Medicine Seminar, 2000

Office of Education, Summer Research Conference, Pre-Clinical Animal Models in Prostate Cancer Research, 2000

The University of Texas M. D. Anderson Cancer Center "Research Council, 2001

Pancreatic Cancer Workshop, 2001

Grand Rounds, Division of Radiation Oncology, 2001

Angiogenesis MRP, 2001

Patient Conference "Circle of Life" held at the Omni Houston Hotel Westside, 2001

Medical Oncology Fellows Conference, 2001

The Marcus Foundation Luncheon and Tour, 2001

Panel Presentation at the Anderson Network 13th Annual "Living Fully with Cancer", 2002

Grand Rounds, Division of Cancer Medicine, 2002

Angiogenesis Workshop, Society of Biological Therapy, San Diego, CA, 2003

Nanotechnology Summit, Houston, TX, 2004

Chair, Institutional Grand Rounds, 2004

Gillson-Longenbaugh Foundation, 2004

Association of Health Care Journalists, 2006

Supervisory Teaching

Advisory Committees and/or Supervisory Committees

Summer Tutorial Research Experiment, Joyce Philips, The University of Texas-Houston Medical School, Houston, TX, 2001

Summer Tutorial Research Experiment, Sharon Fernandez, The University of Texas-Houston Medical School, Houston, TX, 2001 – 2002

Member, Advisory and Supervisory Committee, G.S.B.S., Catherine Papasakelariou (M.S.) 05/08/02 – 08/15/02

Member, Advisory/Supervisory Committee, G.S.B.S., Ryan Von Lindern, 2002

Member, Advisory/Supervisory Committee, G.S.B.S., Marya McCarty (Ph.D.) 2002

Member, Advisory Committee, G.S.B.S., Thomas Merritt (Ph.D.) 2002

Member, Advisory Committee, G.S.B.S., Sarah Dunlap (Ph.D.) 2004

Member, Advisory/Supervisory Committee, G.S.B.S., George Wang, 2003

Member, Advisory Committee, G.S.B.S., Melanie Dujka (Ph.D.) 2004

Member, Advisory Committee, G.S.B.S., Jayaganesh Natarajan (Ph.D.) 2004

Member, Advisory and Supervisory Committee, G.S.B.S., Claudia Vidal (M.D., Ph.D., 2003-2004

Chair, Advisory Committee, G.S.B.S., Catherine Moya (Ph.D.) 2003 – 2006

Tutorial Supervised, Jennifer Dembinski, 2002

Tutorial Supervised, Catherine Moya, 2002-2003

Member, Advisory Committee, G.S.B.S., Catherine Moya (Ph.D.) 2003 – 2006

Tutorial Supervised, Dawn Christianson, 2004

Chair, Advisory Committee, G.S.B.S., Dawn Christianson (Ph.D.) 05/19/04 – Present

Arap/Pasqualini Laboratory Summer Research Program, Tracey Smith, Baylor University, Summer 2005

Arap/Pasqualini Laboratory Summer Research Program, Alicia L. Patterson, Massachusetts Institute of Technology, Summer 2006

Arap/Pasqualini Laboratory Summer Research Program, Shannon Nees, Massachusetts Institute of Technology, Summer 2006

Arap/Pasqualini Laboratory Summer Research Program, Jeffrey A. Easley, Massachusetts Institute of Technology, Summer 2006

Arap/Pasqualini Laboratory Summer Research Program, Julianna K. Edwards, Massachusetts Institute of Technology, Summer 2006

Arap/Pasqualini Laboratory Summer Research Program, Tracey Smith, Baylor University, Summer 2006

Arap/Pasqualini Laboratory Visiting Student, Laura Lattanzio, Institute for Cancer Research and Treatment, University of Turin Medical School, Candiolo, Italy, 2006

Supervisory/Advisory Committee, George Wang, M.D., Ph.D., University of Texas, 2006

Supervisory/Advisory Committee, Colby Suire, Ph.D., University of Texas, 2007

Examining Committee Participation

Chair, Claudia Vidal, 05/03/02

Chair, Catherine Moya, 02/20/06

Supervisory Committees from Other Institutes

Goran Mason, B.S., Graduate Student, Karolinska Institute, Sweden, 1998

Gordon Tang, M.D., Neurosurgery Fellow, Harvard Medical School, Boston, MA, 1998

Bradley Restel, B.S., Research Technician, Medical Student, University of Texas-San Antonio, San Antonio, TX, 1999 – 2001

Carlotta Cavazos, B. S., Research Technician, Physician Assistant School; Baylor College of Medicine, Houston, TX, 1999 – 2001

Visiting Graduate Student, Olaf Broders, University of Heidelberg, Germany, 2000

Visiting Graduate Student, Margaret Magdesian, University of Sao Paulo, Sao Paulo, Brazil, 2000

Visiting Graduate Student, Mark LaBarge, Stanford University, Stanford, CA, 2001

Undergraduate Tutorial, Katherine Leskin, MIT, Summers 2003 and 2004

Johanna Lahdenranta, The University of Helsinki, Finland, 2003

Marina Cardó-Vila, University of Barcelona, Spain, 2003

Graduate Student Tutorial, Fernanda Staquicini, University of Sao Paulo, Brazil, 2004

Graduate Student Tutorial, Michael Stefandakis, University of Helsinki, Finland, 2004

Katja Karjalainen, University of Helsinki, Finland, 2007

Direct Supervision

Undergraduate and Allied Health Students

N/A

Medical Students

Claudia Vidal, M.D., Ph.D. Program, 2002 – 2004, Surgical Resident, University of Pennsylvania

Michael Ozawa, M.D., Ph.D. Program, 2005 - present

Graduate Students

Marina Cardó-Vila, graduated 2003

Catherine Moya, 2003 – 2005

Johanna Lahdenranta, 1998 - 2004, graduated 2004

Dawn Christianson, 2004 – Present

Jessica Sun, 2005 – Present

Alessandro Kelien Lee, 2006 – Present

Tracey Smith, 2006 – Present

Danielle O'Connell, 2007 - Present

Katja Karjalainen, 2007 – Present

Postdoctoral Research Fellows

Virginia Yao, Ph.D., Postdoctoral Fellow, Senior Research Fellow, University of California, San Francisco, CA, 1999 – 2001

Paul Mintz, Ph.D., Postdoctoral Fellow, 1999 – 2003
Instructor, The University of Texas M. D. Anderson Cancer Center, 2003-2006
Associate Professor, Imperial College, UK, 2006-present

Martin Trepel, M.D., Postdoctoral Fellow, Assistant Professor, University of Freiburg, Germany, 2000 – 2001

Peter Ardelt, M.D., Postdoctoral Fellow, Urology Resident, Forschungszentrum Borstel, Germany, 2000 – 2002

Limor Chen, Ph.D., Postdoctoral Fellow, Senior Research Scientist, Dr. Robert Kerbel's Laboratory, Canada, 2000 – 2003

Mikhail Kolonin, Ph.D., Postdoctoral Fellow, 2000 – 2003
Instructor, The University of Texas M. D. Anderson Cancer Center, 2003-2007
Assistant Professor, Institute for Molecular Medicine, Houston, TX, 2007-present

Amado Zurita, M.D., Postdoctoral Fellow, 2001 – 2007
Assistant Professor, The University of Texas M. D. Anderson Cancer Center, 2007-present

Marco Arap, M.D., Postdoctoral Fellow, 2002 – 2003
Assistant Attending Physician and Assistant Professor of Urology USP, 2003 - present

Laura Bover, Ph.D., Postdoctoral Fellow, 2003 – 2004, Research Scientist Immunology

Marina Cardó-Vila, Ph.D., Postdoctoral Fellow, 2003 – Present
Susan G. Komen Fellow, 2006-2009

Glauco Souza, Ph.D., Postdoctoral Fellow, 2003 – Present
Recipient of The Jay and Lori Eisenberg Endowed Fellowship
Recipient of The Marion D. Edwards Fellowship

Diana Jaalouk, Ph.D., Postdoctoral Fellow, 2003 – 2006
Recipient of the Kimberly Patterson Fellowship in Leukemia Research Award, 2006

Ricardo Giordano, Ph.D., Postdoctoral Fellow, 2004 – 2007
Instructor, UTMDACC, 2007-present

Liliana Guzman-Rojas, Ph.D., Postdoctoral Fellow, 2004 – Present

Johanna Lahdenranta, Ph.D., Postdoctoral Fellow, Harvard Medical School, 2004 – 2005

Roberto Rangel, Ph.D., Postdoctoral Fellow, 2004 – Present
Dr. Rangel is the recipient of the Scientific Achievement Fund for Odyssey Fellowship, 2006 – 2008

Fernanda Staquicini, Postdoctoral Fellow, 2004 – Present

Diana Noronha Nunes, Postdoctoral Fellow, 2006 – Present

Jami Mandelin, Postdoctoral Fellow, 2006 – Present

Jaesung Kim, Postdoctoral Fellow, 2007 – Present

Masanori Sato, Postdoctoral Fellow, 2007 – Present

Kisu Kim, Postdoctoral Fellow, 2007 – Present

Wouter Driessen, Postdoctoral Fellow, 2007 – Present

Clinical Residents and Fellows/Faculty
Michael Wang, M.D., Assistant Professor, Department of Lymphoma, The University of Texas M. D. Anderson Cancer Center

Zhiong Lee, M.D., Oncology Practice

Yun Oh, M.D., Assistant Professor, Department of Thoracic/Head and Neck Medical Oncology, The University of Texas M. D. Anderson Cancer Center

Paul Mintz, Ph.D., Instructor, 1999 – Present

Mikhail Kolonin, Ph.D., Instructor, 2003 – Present

Padmanee Sharma, M.D., Ph.D., Assistant Professor, 2004 – 2005

Emmanuel Dias-Neto, Ph.D., Visiting Assistant Professor, 2006 – Present

Erkki Koiuvunen, Ph.D., Visiting Associate Professor, 2007 – Present

Benjamin J. Moeller, M.D., Ph.D., 2007-present

Visiting Scientists

Luisa Villa, Ph.D., Professor, Ludwig Institute, Sao Paulo, Brazil, 02/04 (Sabbatical)

Erkki Koivunen, Ph.D., Associate Professor of Biochemistry, University of Helsinki, Finland, 2000 – 2004

Serena Marchió, Ph.D., Graduate Student, University of Torino, Italy, 2000, 2004

Flavio Curnis, Ph.D., Graduate Student, San Raffaele H. Scientific Institute, Milan, Italy, 2000

Luiz Rizzo, M.D., Ph.D., Associate Professor of Immunology, University of São Paulo, Brazil, 2002 (Sabbatical)

Akihiko Kunyiasu, Associate Professor, Dept. of Biochemistry, Kumamoto University, Kumamoto, Japan, 2003 – 2004 (Sabbatical)

E. Helene Sage, Ph.D., Professor and Director, The Heart Hope Institute, Seattle, 2003, 2004 (Sabbatical)

Emmanuel Dias Neto, Ph.D., Researcher and Deputy-director of the Laboratory of Neurosciences, Instituto de Psiquiatria - Faculdade de Medicina - Univ. de São Paulo, 2005 (Sabbatical)

Erkki Koivunen, Ph.D., Associate Professor of Biochemistry, University of Helsinki, Finland, 2005 (Sabbatical)

Houston Miller, Ph.D., Professor of Chemistry, George Washington University, Washington, DC, 2005 (Sabbatical)

Serena Marchio, Ph.D., Staff Scientist, APAvadis Inc., University of Torino, 2007

Erkki Koivunen, Professor, University of Helsinki, Finland, 2007

Teaching Outside of Current Institution

Formal Teaching

Courses Taught

Instructor, Vascular Biology and Gene Therapy, GSBS, 2002-2005

Instructor, Ph.D. training program in translational bioengineering for cancer diagnostics and therapeutics, Rice University and The University of Texas M. D. Anderson Cancer Center, 2006

CONFERENCES AND SYMPOSIA

Organization of National or International Conferences/Symposia (Including chairing session)

Chair, IBC meeting in Molecular Evolution, Boston, MA, 1997

Chair, Extracellular Matrix and Cell Function, SIMEC, Rio de Janeiro, Brazil, 1998

Chair, ASGT Angiogenesis Educational session, Denver, CO, 2000

Chair, Targeted Therapies, 11th NCI-EORTC-AACR Symposium, 2000

Co-organizer, Chair, Phage Display Technology, CHT meeting, Boston, MA, 2000

Chair, ASGT Angiogenesis Educational session, Seattle, WA, 2001

Scientific Advisory Board, Chair, Phage Display Technology, CHT meeting, Boston, MA, 2001

Chair, Vector Targeting Strategies for Therapeutic Gene Delivery, Cold Spring Harbor, NY, 2001

Chair, Novel Targeted Therapies, AACR, New Orleans, LA, 2001

Chair, Angiogenesis Regulation and Vascular Targeting, 2001

Mini-symposium, AACR, 2001

Chair, Targeted Therapies, AACR-NCI-EORTC meeting, Miami, FL, 2001

Organizing Committee, EORTC-NCI-AACR Symposium, Mol. Targets, Frankfurt, Germany, 2002

Scientific Advisory Board, Phage Display Technology, CHT meeting, Boston, MA, 2002

Chair, 67th Cold Spring Harbor Symposium on Quantitative Biology: The Cardiovascular System, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 2002

Scientific Advisory Board, Phage Display Technology, CHT meeting, Boston, MA, 2003

Chair, Tumor Microenvironment, 94th AACR Annual Meeting, 2003

Chair, CHT Institute, Molecular Display, Boston, 2003

Chair, Tumor Microenvironment, AACR 97th Annual Meeting, 2003

Member, Special Conferences Committee, AACR, 2003-2006

Member, AACR Special Conference Committee, Cancer Proteomics, 2004

Co-Chair, AACR Special Conference: Basic, Translational, and Clinical Advances in the Management of Prostate Cancer, 2004

Co-Chair, Tumor Microenvironment Minisymposium, AACR Annual Meeting, 2004

Member, M. D. Anderson Cancer Center, Sister Institution Conference, South America Partnership Meeting Organizing Committee, 2005/2006

Co-Chair, The 2006 Miami Nature Biotechnology Winter Symposium: Angiogenesis in Cancer and Vascular Diseases, 2006

Member, Scientific Program Committee for the 5th Annual Meeting of the Society for Molecular Imaging, 2006

Co-Chair, AACR Special Conference: Innovations in Prostate Cancer, 2006

Member, Scientific Program Committee for the 6th Annual Meeting of the Society for Molecular Imaging, 2007

Organizing Committee, Session Chair, International Society of Biological Therapy, 2007

Presentations at National or International Conferences
Invited (since relocation to The University of Texas M. D. Anderson Cancer Center)

Cold Spring Harbor, "Vector Targeting Strategies for Therapeutic Gene Delivery, NY, 03/99

Phage Display, Therapeutics and Diagnostics, CHI, Boston, MA, 03/99

American Society of Gene Therapy, Washington DC, MD, 06/99

International Symposium in Radionuclides, St. Louis, MO, 06/99

Signaling and Angiogenesis UICC Course, Tamsvik, Sweden, 08/1999

Gordon Research Conference in Angiogenesis, Salve Regina, RI, 08/99

Gordon Research Conference in Central Nervous System, RI, 08/99

Grantee National Meeting, The Susan G. Komen Foundation, Dallas, TX, 10/99

Course on Phage Antibodies, Cold Spring Harbor Laboratories, CSH, NY, 11/99

International Symposium in Angiogenesis, Milan, Italy, 11/99

Novartis Symposium in Vascular Biology, Basel, Switzerland, 11/99

Therapeutic Control of Angiogenesis, London, UK, 01/00

Gordon Research Conference, Drug Delivery, Ventura, CA, 02/00

Human Gene Therapy Course, The University of Texas M. D. Anderson Cancer Center, Houston, TX, 02/00

Clinical and Biological Aspects of Urothelial Cancer, Houston, TX, 03/00

International Meeting on Angiogenesis, Experimental and Clinical, The Netherlands, 04/00

Phage Display, Diagnostics and Therapeutics, CHI, Boston, MA, 04/00

FASEB Symposium on Angiogenesis and Signaling, San Diego, CA, 04/00

Swedish Society of Oncology, Nobel Forum, Karolinska Institute, Stockholm, Sweden, 05/00

American Society of Gene Therapy 3rd Annual Meeting, Denver, CO, 05/00

National Heart, Lung, and Blood Institute, Meeting on Vascular Heterogeneity, 06/00

UTOPIA Workshop, Heidelberg, Germany, 07/00

American Urological Association, 07/00

International Symposium on Apoptosis, Caxambu, Brazil, 08/00

26th European Peptide Symposium, Montpellier, France, 09/00

Understanding Phage Display: Structure, Biology and Applications, Vancouver, Canada, 09/00

Metastasis Research Society, London, UK, 09/00

International Meeting on Angiogenesis, Montreal, Canada, 10/00

11th NCI-EORTC-AACR Symposium, The Netherlands, 11/00

Josef Steiner Foundation Symposium, Basel, Switzerland, 01/01

Euroconference in Angiogenesis, Paris, France, 03/01

American Association for Cancer Research Annual Meeting, New Orleans, LA, 03/01

Phage Display, Diagnostics and Therapeutics, CHI, Boston, MA, 04/01

European Meeting in Angiogenesis, Liege, Belgium, 04/01

International Course on Angiogenesis and Signal Transduction, Sicily, Italy, 04/01

Angiogenesis I & II, American Society of Gene Therapy, Seattle, WA, 05/01

Controlled Release Society Annual Meeting, San Diego, CA, 06/01

Macromolecular Drug Delivery Conference, Breckenridge, CO, 07/01

International Conference on Angiogenesis and Tumors, Paris, France, 09/01

INSERM Collège de France, Paris, France, 09/01

Imaging in 2020 Conference, WY, 09/01

Cancer Research Institute Symposium, Antibodies in Cancer, New York, NY, 10/01

Forbeck Meeting in Vascular Permeability, Napa Valley, CA, 10/01

AACR-NCI-EORTC International Conference, Miami, FL, 10/01

Cold Spring Harbor Laboratory Course on Phage Display of Combinatorial Antibody Libraries, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 11/01

International Meeting in Molecular Imaging, Orlando, FL, 01/02

Annual Genitourinary Oncology Conference, The University of Texas M. D. Anderson Cancer Center, Houston, TX, 02/02

NCI, Vascular Biology Faculty Retreat, Washington, DC, 02/02

Division of Cancer Treatment and Diagnosis, NCI, Annapolis, MD, 03/02

American Association for Cancer Research 93rd Annual Meeting, San Francisco, CA, 04/02

Tumor Microenvironment: Biology and Therapeutic Implications, Round Top, TX, 04/02

67th Cold Spring Harbor Symposium on Quantitative Biology: The Cardiovascular System, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 05/02

Middle East Medical Assembly, Beirut, Lebanon, 05/02

Liposome Research Days Inc. - MDC, Berlin, Germany, 05/02

Novo Nordisk Foundation Consortium Symposium, Upplands Vasby, Sweden, 05/02

Symposium on Angiogenesis in Oncology and Hematology, Muenster, Germany, 07/02

NCI-Interdisciplinary Research Teams for Molecular Target Assessment, Seattle, WA, 08/02

1st International Congress on Targeted Therapies, Washington, DC, 08/02

Department of Defense, Breast Cancer Research Program Meeting, Orlando, Florida, 10/02

Brain Endothelium and Pathologies, ISERM, Paris, France, 10/02

American Society for Extracellular Matrix, Houston, TX, 11/02

International Symposium on Head and Neck Cancer, Puerto Rico, 01/03

U.S. – Japan Workshop, "Tumor-Specific Delivery by Non-Viral Systems: Approaching a Reality," Maui, HI, 02/03

U.S. - Japan Cooperative Medical Science Program, Environment Genomics and Carcinogenesis Panel, Kyoto, Japan, 03/03

American Association for Cancer Research 97th Annual Meeting, Washington DC, 04/03

2nd European Society of Combinatorial Sciences (ESCS) Society Symposium, Copenhagen, Denmark, 06/03

4th Symposium on the Biology of Endothelial Cells, Munich, Germany, 07/03

International Meeting, "How Close Are We From Cancer," São Paulo, Brazil, 08/03

Gordon Research Conference: Angiogenesis and Microcirculation, Newport, RI, 08/03

Wenner-Gren Foundation International Symposium, Biology of Tumor Stroma: Potential Avenues in Tumor Therapy, Stockholm, Sweden, 09/03

Molecular Targets NCI Workshop, Philadelphia, PA, 10/03

Ludwig Institute Cancer Center, London, England, 10/03

Vanderbilt-Ingram Cancer Center Proposal for Proteomics-Bases Collaboration: Use of Circulating Antibodies to Discover Tumor Antigens, Nashville, TN, 10/03

U01 Mini Workshop, Contrasting Properties of Integrin Cytoplasmic Domains, Philadelphia, PA, 10/03

European School of Hematology/The University of Texas M. D. Anderson Cancer Center Conference: Mechanisms of Cell Death and Disease: Advances in Therapeutic Interventions, Cancun, Mexico, 11/03

NCI, Frederick, Maryland, 11/03

Phage Display of Combinatorial Antibody Libraries at Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 11/03

Department of Defense, Generals Meeting on Homeland Security, Baltimore, MD, 12/03

Institutional Grand Rounds, The University of Texas M. D. Anderson Cancer Center, Houston, TX, 02/04

Cellular, Molecular, and Tumor Biology, 95th Annual AACR Meeting, Orlando, FL, 03/04

Mayo Oncology Society, April Oncology Society Presentation, Rochester, MN, 04/04

Alliance for NanoHealth Workshop, Texas Heart Institute, Denton Cooley Auditorium, Houston, TX, 05/04

Thomas L. Petty Aspen Lung Conference 47th Annual Meeting on Cellular and Molecular Pathobiology of Pulmonary Hypertension, Aspen, CO, 06/04

32nd Meeting of the International Society for Oncodevelopmental Biology and Medicine (ISOBM), Helsinki, Finland, 06/04

Endothelial Cell Phenotypes in Health and Disease Gordon Research Conference, 07/04

12th SPORE Investigator's Workshop, Baltimore, MD, 07/04

Winship Cancer Institute, Emory University School of Medicine, Atlanta, GA, 08/04

Gordon Research Conference, Endothelial Cell Phenotypes in Health & Disease, Andover, NH, 08/04

Grover Conference on the Pulmonary Circulation: Genetic and Environmental Determinants of Pulmonary Endothelial Cell Function, Sedalia, CO, 09/04

3rd International Symposium on Extracellular Matrix (SIMEC), Rio de Janeiro, Brazil, 09/04

2nd "Biologie Prospective" Santorini Conference, Santorini, Greece, 09/04

1st European Conference on Tumor Angiogenesis and Antiangiogenic Therapy, Munich, Germany, 10/04

NIH CIP Workshop on High-Throughput Technologies, Washington, DC, 11/04

AACR: Basic, Translational, and Clinical Advances in Prostate Cancer, Bonita Springs, FL, 11/04

Symposium New Therapies in Cancer, Centro Nacional de Investigaciones Oncologicas, Madrid, Spain, 11/04

Imperial College, London, England, 11/04

Gordon Research Conference: Fibronectin, Integrins & Related Molecules, Ventura, CA, 01/05

AACR Special Conference Committee Meeting, Miami Beach, FL, 01/05

Advances in Oncology Institutional Grand Rounds, The University of Texas M. D. Anderson Cancer Center, Houston, TX, 01/05

National Heart, Lung and Blood Institute, Bethesda, MD, 04/05

Liver Cancer Institute, Fudan University, Shanghai, China, 05/05

Research Centre of Cancer, Faculty of Medicine, The University of Hong Kong, Hong Kong, 05/05

12th Annual Scientific Retreat, Prostate Cancer Foundation, Phoenix, AZ, 09/05

Department of Biological and Technological Research of San Raffaele H Scientific Institute, Milan, Italy, 10/05

Institute for Cancer Research and Treatment, Candiolo, Torino, Italy, 10/05

Annual Advisory Board Meeting of the Prostate Cancer Research Program, Houston, TX, 11/05

6th Peter MacCallum Cancer Symposium, Melbourne, Australia, 11/05

National Cancer Center Research Institute, Tokyo, Japan, 12/05

Sapporo Medical University School of Medicine, Sapporo, Japan, 12/05

Speaker, 2006 Miami Nature Biotechnology Winter Symposium, Days of Molecular Medicine: Angiogenesis in Cancer and Vascular Disease, Miami, FL, 02/06

Speaker, Present Project 7 Progress, Houston, TX, 02/06

Speaker, The University of São Paulo, Radiation Oncology Program, São Paulo, Brazil, 3/06

Speaker, Association of Health Care Journalists Conference, Houston, TX, 03/06

Speaker, Bioengineering Graduate Course, Rice University, Houston, TX, 03/06

Speaker, The University of Kumamoto, Kumamoto, Japan, 04/06

Speaker, Department of Cellular and Integrative Physiology Seminar, Indianapolis, IN, 04/06

Speaker, The MDACC-Severance Symposium 2006, Seoul, Korea, 05/06

PMT Distinguished Seminar Speaker, University of North Carolina, Chapel Hill, NC, 05/06

Speaker, Rockefeller University, New York, NY, 05/06

Speaker, BD Technologies, Research Triangle Park, NC, 05/06

Speaker, 2nd Annual M. D. Anderson Cancer Center Sister Institution Conference, Houston, TX, 06/06

Invited Speaker, Research Conference, Department of Lymphoma/Myeloma, The University of Texas M. D. Anderson Cancer Center, Houston, TX, 06/06

Invited Speaker, Division of Cancer Medicine, The University of Texas M. D. Anderson Cancer Center, Houston, TX, 07/06

Chair, The University of Texas M. D. Anderson Cancer Center Grand Rounds, Houston, TX, 07/06

Speaker, 14th Spore Investigator's Workshop, Baltimore, MD, 07/06

Speaker, Aspen Seminar and Hines Reception, Aspen, CO, 07/06

Speaker, The V Foundation Awardees Annual meeting, Napa Valley, CA 07/06

Invited Speaker, Department of Bioengineering, Rice University, Houston, TX, 08/06

Speaker, IGR-MDACC International Scientific Symposium/Sister Institution, Extramural Program, Paris, France, 09/06

Speaker, 4th Annual Angiogenesis & Vascular Targeting Agents Drug Discovery & Development World Summit, Boston, MA, 09/06

Speaker, Bastrop Veterinarian Team Meeting; GLP Pre-Clinical Work for Anticancer Peptides Bastrop, TX, 09/06

Speaker, Prostate Cancer Foundation 13th Annual Scientific Retreat, Scottsdale, AZ, 10/06

Speaker, Diagnosis and Therapeutic Discovery in Neuro-Oncology Conference, Houston, TX, 10/06

Speaker, Clontech, Mountain View, CA, 10/06

Speaker, A.C. Camargo/Sister Institution, Extramural Program, São Paulo, Brazil, 10/06

Speaker, Biogen, Boston, MA, 11/06

Speaker, 18th EORTC-NCI-AACR Symposium, Prague, Czech Republic, 11/06

Speaker, 6th Edition of Amazon Project Conference on Cancer, Palermo, Italy, 11/06

Speaker, San Rafaelle Hospital, Milan, Italy, 11/06

Speaker, Arap/Pasqualini Program Retreat, San Francisco, CA, 12/06

Speaker, University of Texas M. D. Anderson Cancer Center, Grand Rounds, Houston, TX, 01/07

Speaker, Nano Medicine Annual Symposium, Helsinki, Finland, 01/07

Speaker, International Symposium on Polymer Therapeutics (ISPT 07), Berlin, Germany, 02/07

Speaker, 2007 Advances in Oncology: Emerging Trends, Targets, and Approaches to Solid Tumors Symposium, Houston, TX, 03/07

Speaker, Memorial-Sloan Kettering Cancer Center, Molecular Pharmacology & Chemistry Seminar, New York, NY, 03/07

Speaker, Steele Laboratory Interactive Tumor Biology Seminar Series, Massachusetts General Hospital, Boston, MA, 04/07

Speaker, Stanford University, Palo Alto, CA, 04/07

Speaker, McGill Cancer Centre, Montreal, Canada, 04/07

Speaker, Imaging Short Course, Rice University, Houston, TX, 05/21/07

Speaker, 2007 Days of Molecular Medicine, Emerging Technologies and Cancer Biology Symposium, Cambridge, MA, 05/21/07–05/23/07

Speaker, BIT's 5th Anniversary, The Conference and Expo of International Drug Discovery Science and Technology (IDDST), China Roles for Global Drug Innovations, Shanghai, China, 05/27/07-05/31/07

Speaker, Annual Meeting of the European Society for Molecular Imaging, Naples, Italy, 06/14/07-06/15/07

Speaker, Michale E. Keeling Center for Comparative Medicine and Research, Department of Veterinary Sciences, Bastrop, TX, 07/20/07-07/24/07

Speaker, 13th Annual International Congress of Immunology, Rio de Janeiro, Brazil, 08/20/07-08/25/07

Speaker, U.S. Army Institute of Surgical Research, Fort Sam Houston, San Antonio, TX 08/28/07

Speaker, USC Grand Rounds, USC Norris Cancer Center, Los Angeles, CA 09/10/07-09/11/07

Speaker, Conway Institute, University College of Dublin, Dublin, Ireland 09/24/07-09/28/07

Speaker, 2007 Prostate Cancer Foundation Scientific Retreat, Lake Tahoe, NV, 10/11/07-10/13/07

Speaker, 2nd Annual France – USA Science & Technology Workshop, Rice University, Houston, TX, 10/15/07

Speaker, TATRC's Integrated Research Team (IRT) Meeting, Nanotechnology Solutions for Long-term Implantable Devices, The University of Texas Health Science Center, Houston, TX, 10/24/07

International Symposium "Mechanisms of Tumor Control", Sao Paulo, Brazil, 11/07

Other, Including Scientific Exhibitions

Aventis, Frankfurt, Germany, 7/00

Elan Corporation, Dublin, Ireland, 09/00

Aventis, San Jose, CA, 02/01

Ely Lilly Corporation, Indianapolis, IN, 10/01

AstraZeneca, Waltham, MA, 12/01

Becton Dickson, North Carolina, 01/03

Novartis, San Diego, 01/03

Abgenix, San Francisco, 03/03

Texas Academy of Science, Engineering and Medicine (Academy) Inaugural Conference, 01/04

Abgenix, Bunaby BC, Canada, 01/04

Pfizer Global Research and Development Seminar, San Diego, CA, 04/05

BD Technologies, Research Triangle Park, NC, 05/06

Clontech, Mountain View, CA, 10/06

Biogen, Boston, MA, 11/06

Seminar Invitations from Other Institutions

University of Texas Southwestern, Dallas, TX, 1999

Vascular Biology Seminar, Harvard Medical School, Boston, MA, 1999

Cell and Molecular Biology Seminar, MGH, Boston, MA, 1999

Sidney Kimmel Cancer Center, La Jolla, CA, 1999

Gene Therapy Program, University of California-San Diego, CA, 1999

Seminar Series, Wayne University, Detroit, MI, 1999

Seminar Series, University of Missouri-Columbia, Columbia, MO, 1999

FMI, Basel, Switzerland, 1999

Seminar Series, University of Torino, Torino, Italy, 1999

University of California-Los Angeles Seminar Series, Los Angeles, CA, 2000

Seminar Series, Yale University School of Medicine, New Haven, CT, 2000

Molecular & Cellular Oncology Seminar, 2000

Oncology Seminar Series, DFCI, Harvard Medical School, Boston, MA, 2001

Seminar Series, Moffitt Cancer Center, University of South Florida, Tampa, FL, 2001

Stanford Angiogenesis Research Forum, Stanford University, Stanford, CA, 2001

University of Pennsylvania Seminar Series, Philadelphia, PA, 2002

The Cleveland Clinic Foundation, The Lerner Research Institute, Department of Cancer Biology Seminar Series, Cleveland, OH, 2002

Nanotechnology Symposium, Rice University, Houston, TX, 2003

Molecular Targets NCI Workshop, Philadelphia, PA, 2003

National Cancer Institute Seminar Series, Frederick, MD, 2003

The Vanderbilt-Ingram Cancer Center, Nashville, TN, 2003

Johns Hopkins Medical Institute, Baltimore, MD, 2003

Universitat de Barcelona, Barcelona, Spain, 2003

University of California-San Francisco, San Francisco, CA, 2003

Baylor Gene Therapy Seminar Series, Houston, TX, 2004

Oncology Society, Rochester, MN, 2004

Nanohealth Summit, Houston, TX, 2004

NCI Seminar Series, Immunotherapy, 2004

Vascular Biology Seminar Series, Boston, MA, 02/04

Vari Seminar Series, Grand Rapids, Michigan, 03/04

Baylor College of Medicine Gene Therapy Seminar Series, 05/04

Van Andel Research Institute Seminar Series, Grand Rapids, MI, 08/04

Winship Cancer Institute, Emory University School of Medicine, Atlanta, GA, 08/04

Workshop on Biomedical Sensing and Imaging to the Nano-scale, Texas A&M University, College Station, TX, 10/04

Cancer Biology & Genetic Seminar, Memorial Sloan-Kettering Cancer Center Institute, New York, NY, 12/04

XXXV International Congress of Physiological Sciences Congress Symposium, San Diego, CA, 04/05

Henderson Research Centre Seminar, Hamilton, Canada, 04/05

Anti-Angiogenesis in Oncology Seminar, Pfizer Global Research and Development Seminar, San Diego, CA, 04/05

University of Michigan Life Sciences Institute Fourth Annual Symposium, Cancer Insights: Molecules to Medicine, Ann Arbor, MI, 05/05

Cancer Center Seminar Series, The City of Hope Comprehensive Cancer Center and Beckman Research Institute of the City of Hope, Duarte, CA, 06/05

Center for Cell Biology & Cancer Research, Albany Medical College, Albany, NY, 09/05

Boston Children's Hospital/Harvard Medical School, Boston, MA, 09/05

University of Michigan, Cancer Focus Group, Ann Arbor, MI, 10/05

Massachusetts Institute of Technology, Cambridge, MA, 03/06

Winship Cancer Institute, Emory University School of Medicine, Atlanta, GA, 04/06

PMT Distinguished Seminar Speaker, University of North Carolina, Chapel Hill, NC, 05/06

Lectureships and Visiting Professorships

Angiogenesis Course, University of Nebraska-Lincoln, Lincoln, NE, 2000

2nd International Symposium in Gene Therapy, Institute of Life Science of Yonsei University, Seoul, Korea, 2001

Cold Spring Harbor, "Vector Targeting Strategies" New York, NY, 2001

Other Presentations at State and Local Conferences

N/A

PROFESSIONAL MEMBERSHIPS/ACTIVITIES

Professional Society Activities, with Offices Held

National and International

Member, European Academy of Sciences

Member, American Society for Biochemistry and Molecular Biology (ASBMB), 2003

Member, Federation of American Societies for Experimental Biology (FASEB), 2003

Member, American Association for Cancer Research (AACR)

Member, American Association for the Advancement of Science

Member, American Society for Cell Biology

Member, PanAmerican Association for Biological Sciences

Member, American Society for Cancer Research

Member, American Society of Gene Therapy

Member, Society for Molecular Imaging

Local/state

N/A

OTHER

Guest Lecturers hosted:

Dr. Linda Shapiro, Associate Professor, St. Judes Children's Hospital, Memphis, TN, Genitourinary Oncology Research Seminar Series, 10/00

Dr. Rubin Tuder, Associate Professor of Pathology, Johns Hopkins University, Baltimore, MD, Research Seminar Series, 9/01

Dr. Neil Pellis, Director of Research, Johnson's Space Center, NASA Houston, TX, Research Seminar Series, 9/01

Dr. Bruce Zetter, Professor of Cancer Biology, Harvard Medical School, Children's Hospital, Boston, MA, Genitourinary Oncology Research Seminar Series, 10/01

Dr. Steven K. Libutti, Senior Investigator, Surgery Branch, NCI, Bethesda, MD, Blaffer/Keck Seminar Series in Virology and Gene Therapy, 10/01

Dr. E. Helene Sage, Director, Division of Basic Science, The Hope Heart Institute, Seattle, WA, Genitourinary Oncology Research Seminar Series, 11/01

Dr. Donald McDonald, Professor of Anatomy, Cardiovascular Research Institute, University of California, San Francisco, CA, Genitourinary Oncology Research Seminar Series, 12/01

Dr. David A. Cheresh, Professor, Departments of Immunology and Vascular Biology, The Scripps Research Institute, La Jolla, CA, Genitourinary Oncology Research Seminar Series, 01/02

Dr. Houston Miller and Dr. Glauco Souza, George Washington University, Washington, DC, Genitourinary Oncology Research Seminar Series, 01/02

Dr. Richard C. Mulligan, Mallinckrodt Professor of Genetics, Department of Genetics, Harvard Medical School, Boston, MA, Blaffer/Keck Seminar Series in Virology and Gene Therapy, 04/02

Dr. Leonard A. Herzenberg, Professor of Genetics, Emeritus, Department of Genetics, Stanford University School of Medicine, Stanford, CA, Blaffer/Keck Seminar Series in Virology and Gene Therapy, 06/02

Dr. Leonore A. Herzenberg, Professor of Research, Department of Genetics, Stanford University School of Medicine, Stanford, CA, Genitourinary Oncology Research Seminar Series, 06/02

Dr. Sergio Lira, Director, Department of Immunology, Schering-Plough Research Institute, Kenilworth, NJ, Department of Cancer Biology Seminar Series, 06/02

Dr. Raghu Kalluri, Associate Professor, Beth Israel-Deaconess, Harvard Medical School, Boston, MA, 9/02

Dr. Luisa Villa, Director, Department of Virology, The Ludwig Institute for Cancer Research, Brazil, 10/03

Dr. Luiz V. Rizzo, Associate Professor, The Heart Institute, University of São Paulo, Brazil, 10/03

Dr. Lisa Coussens, Associate Professor, University of California/San Francisco, San Francisco, CA, 10/03

Dr. Pierre-Olivier Couraud, Professor, ISERM, France, 11/03

Dr. Kevin Burgess, Professor, Texas A & M University, College Station, TX, 02/04

Dr. John Reed, President and CEO, The Burnham Institute, La Jolla, CA, 03/04

Dr. Kristina Vuori, Deputy Director, The Burnham Institute, La Jolla, CA, 03/04

Dr. Gilbert D. Loria Masis, Professor, University of Costa Rica, Department of Virology, School of Microbiology, San Jose, Costa Rica, 04/04

Dr. Amy Lee, Associate Director of Basic Research, USC/Norris Cancer Center, Los Angeles. CA, 05/04

Dr. Ricardo R. Brentani, President and CEO, The Ludwig Institute, São Paulo, Brazil, 06/04

Dr. Emmanuel Dias Neto, Researcher and Deputy-director of the Laboratory of Neurosciences, Instituto de Psiquiatria - Faculdade de Medicina - Univ. de São Paulo, 07/04

Dr. Lu Shan, Chemical Engineer, Dept. of Chemical Engineering, Stanford University, Stanford, CA, 07/04

Dr. Ricardo R. Brentani, President and CEO, The Ludwig Institute, São Paulo, Brazil, 07/04

Dr. Fabio C.L. Almeida, Associate Professor/Professor Adjunct, Federal University, Rio de Janeiro, Brazil, 09/04

Dr. Theresa Mary Allen, Professor, Department of Pharmacology, University of Alberta School of Medicine, Edmonton, Alberta, Canada, 03/05

Dr. Tong-Young Lee, Postdoctoral Fellow, Institute of Pathology, College of Medicine, National Taiwan University, Taipei, Taiwan, 04/05

Dr. Claudio A. Joazeiro, Group Leader, The Genomics Institute of the Novartis Foundation, San Diego, CA, 04/05

Dr. Keith L. March, Associate Professor, Department of Cellular and Integrative Physiology, Indiana University, Director, Indiana Center for Vascular Biology and Medicine, Associate Professor, Department of Biomedical Engineering, Purdue University, Indianapolis, IN, 06/05

Dr. Thomas C. Killian, Assistant Professor, Department of Physics and Astronomy, Rice University, Houston, TX, 9/05

Dr. Rebecca Rae Richards-Kortum, Professor, Department of Bioengineering, Rice University, Houston, TX, 10/05

Drs. Brian Freeman and Andrew Perlman, Great Point Ventures, Boston, MA, 10/05

Drs. Henrietta Kulaga and Jon Mogford, DARPA, Arlington, VA, 10/05

Dr. Paul J. Simmons, NHMRC Senior Research Fellow, Program Head in Stem Cell Biology, Director, Adult Stem Cell Platform, Peter MacCallum Cancer Centre, Stem Cell Centre, Melbourne, Australia, 12/05

Dr. Angela Papageorgiou, M.D. Anderson Cancer Center, Department of Cancer Biology, Houston, TX, 12/05

Alessandro Kelien Lee, Laboratory of Molecular Angiogenesis, IRCC – Institute of Cancer Research and Treatment, Candiolo (TO), Italy

Brain Tumor Center Special Seminar invited speaker, Dr. Mark Noble, University of Rochester, School of Medicine and Dentistry, Rochester, NY, 12/05

John H. Blaffer Lecture Series invited speaker, Dr. Robert Benezra, Memorial Sloan-Kettering Cancer Center, New York, NY, 12/05

Kris C. Wood, Ph.D. Candidate, Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, 03/06

Dr. Chae-Ok Yun, Associate Professor, Institute for Cancer Research, Yonsei Cancer Center, Yonsei University of College of Medicine, Seoul, Korea, 03/06

ERO Blaffer Visiting Professorship Lecture invited speaker, Dr. Richard Kolesnick, Head of the Laboratory of Signal Transduction of the Sloan-Kettering Institute, New York, NY, 03/06

Dr. Alberto Bardelli, Associate Professor, Department of Oncological Sciences, University of Torino, School of Medicine, Torino, Italy, 03/06

Dr. Russell L. Finley, Jr., Associate Professor, Center for Molecular Medicine and Genetics, and Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, MI, 03/06

Dr. Erik Henke, Research Fellow in Dr. Robert Benezra's Laboratory, Department of Cancer Biology and Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY, 05/06

Dr. Masanori Soto, Staff Scientist, Biology Division, National Cancer Center Research Institute, Tokyo, Japan, 05/06

Dr. Satoshi Kawaguichi, Department of Orthopedic Surgery, Sapporo Medical University School of Medicine, Sapporo, Japan, 06/06

Dr. Angelo Corti, Head of Tumor Biology and Vascular Targeting Unit, DIBIT-San Raffaele H Scientific Institute, Milan, Italy, 08/06

Dr. Robin L. Anderson, Head, Cancer Biology Laboratory, Peter MacCallum Cancer Centre, Melbourne, Australia, 08/06

Eric Berger, Science Writer, Houston Chronicle, 08/06

Dr. Jaesung Kim, Institute for Cancer Research, Yonsei Cancer Center, Yonsei University College of Medicine, Republic of Korea, Seoul, Korea, 09/06

Laura Lattanzio, Institute for Cancer Research and Treatment, University of Turin Medical School, Candiolo, Italy, 09/06

Dr. Randy J. Seeley, PhD, Department of Psychiatry, Genome Research Institute, University of Cincinnati, Cincinnati, OH, 04/07

Dr. E. Helene Sage, PhD, Member and Director, Hope Heart Program, Benaroya Research Institute at Virginia Mason, Seattle, WA, 05/07

Dr. Joao Setubal, Associate Professor of Bioinformatics, Virginia Tech, Virginia, VA, 10/07

Dr. Patrick Kee, University of Texas, Houston, TX, 11/07

Just as clinical trials of a widely heralded cancer treatment are about to be expanded, two groups report that they couldn't get it to work, indicating again how fickle and mysterious the compound remains

Setbacks for Endostatin

Harvard University's Judah Folkman electrified cancer researchers 5 years ago when he and his colleagues reported on a new compound that could shrink tumors in mice virtually to nothing. A surgeon at Children's Hospital Boston, Folkman had long pursued a strategy of fighting cancer by cutting off the blood supply to tumors, rather than by poisoning patients with toxic drugs. Using a substance called endostatin, the Harvard group obtained dramatic results; clinical trials soon followed. But some other researchers who attempted to follow this lead were unable to find endostatin's seemingly miraculous properties. Now two new studies, published in the April issue of *Molecular Therapy*, take aim at endostatin again, both reporting that it had no effect on tumors in mice.

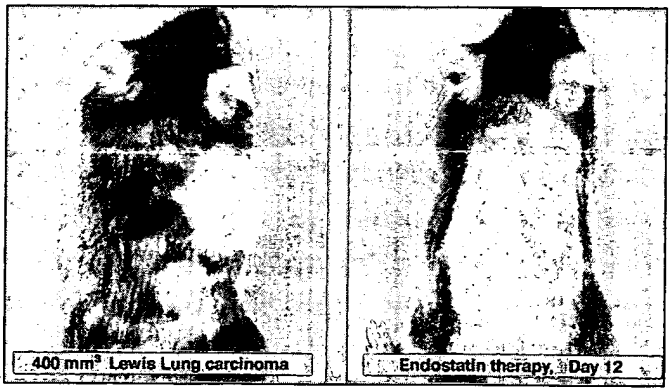
Although these papers are not the first to raise questions about endostatin, they are among the most pointed. One title speaks of "the unfulfilled promise of endostatin" in a type of gene therapy for mice with leukemia. And the other reports that, "despite continuous, high-level secretion of endostatin" in the bloodstream of mice, "we detected neither inhibition of [blood vessel growth] nor antitumor activity." In a companion essay, *Molecular Therapy*'s editor, Fintan Steele, writes that "results from these two groups certainly contradict much of what has appeared in prior publications." The confusion about which data are reliable prompts Steele to ask whether there is "sufficient basic science to understand what endostatin is and what it does"—and whether it makes sense to expand clinical trials built upon the early reports.

Folkman and Michael O'Reilly, the researcher in his lab who discovered endostatin, see no reason to pause. Although Folkman acknowledges that some gene transfer experiments such as those reported in *Molecular Therapy* have not worked out, he says others have been more promising. He and O'Reilly, who is now at the M. D. Anderson Cancer Center in Houston, Texas, also argue that the simpler approach of injecting endostatin directly has yielded positive results in animals that justify expanding clinical trials. So far, fewer than 200 patients have taken part in tests designed to measure safety. No cures were expected, and none have been reported.

Conscious of endostatin's mixed record, some leaders in this field agree that the picture is not as simple as it seemed 5 years ago. As Robert Kerbel of the University of Toronto says, the pharmacokinetics of compounds designed to prevent blood vessel growth (antiangiogenics) may be "very complex," and the method of administration can have a "huge impact" on efficacy. Folkman himself views the complexity as intriguing, adding that even negative reports are useful because they may help unravel the mysteries.

Lapsed believer

When O'Reilly and Folkman first described endostatin in the 24 January 1997 issue of *Cell*, it seemed like an ideal anticancer weapon. This naturally produced, nontoxic compound selectively shrank blood vessels and repeatedly caused tumors in mice to shrink to microscopic size. A later paper in *Nature*, with still more promising results, triggered bold predictions, including a report in *The New York Times* quoting Nobel laureate James Watson to the effect that Folkman would "cure cancer in 2 years." This led to front-page stories and turned Folkman into a reluctant hero. He also became the subject of a popular book, *Dr. Folkman's War*, published last year.



Powerful result. Endostatin therapy dramatically shrank mouse tumors in a 1997 experiment that raised high hopes for antiangiogenesis treatment.

Since then, Folkman's group has expanded its work to other compounds that inhibit blood vessel growth and explored dozens of ideas for new therapies. Many other groups around the world also have plunged in. A private company—EntreMed Inc. of Rockville, Maryland—obtained rights to manufacture endostatin and since the late 1990s has sponsored small clinical trials. The National Cancer Institute (NCI) provided support too, funding a couple of clinical trials and animal studies on endostatin and other antiangiogenics carried out in well-established laboratories. But the two papers in *Molecular Therapy* have raised new red flags, including a report from one lab that it couldn't repeat the original 1997 experiment.

Philippe Leboulch, a contributor to both papers in *Molecular Therapy* and the senior author of one of them, has turned from endostatin enthusiast to skeptic. A molecular geneticist, Leboulch investigates gene therapy techniques with a joint position at the Massachusetts Institute of Technology (MIT) and Harvard Medical School. He also has a small company, Genetix Pharmaceuticals Inc. in Cambridge, Massachusetts. Inspired by early data from Folkman's lab, he embraced endostatin in 1995.

When Leboulch first connected with Folkman's team, he says: "We were very excited about collaborating." One barrier to research in the early days, Leboulch explains, was that endostatin was hard to get. The endostatin for the successful 1997 Folkman lab mouse experiment had been produced in the bacteria *Escherichia coli*. But output was low, and the product was an insoluble aggregate. The MIT group—including Robert Pawliuk, Thomas Bachelot, and Omar Zurkiya—took a different route: This team spliced the endostatin gene into mouse hematopoietic stem cells, the progenitors of blood cells that live in bone marrow. This looked like a great strategy for getting endostatin expressed continuously and at high levels in animals.

Gene transfer worked "as we had planned," recalls Leboulch. "We got very high levels of secretion" in the bloodstream of mice: about 746 nanograms per milliliter (ng/ml), he says. Leboulch estimates that this systemic concentration, on average, was 750% higher than that naturally found in the animals. Eighteen mice received endostatin-expressing stem cells, and 10 received cells that didn't express the protein.

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To look for effects on blood vessel formation, Leboulch collaborated with Yihai Cao of the Karolinska Institute in Stockholm, who is an expert on angiogenesis. Cao compared five endostatin-treated and four control mice and saw no antiangiogenic effect. "In theory, [endostatin gene transfer] should have worked," says Cao. "I don't see why it didn't." He speculates that the protein produced by the transplanted gene may have been misfolded—a possibility Leboulch concedes. But no one knows how the active form of endostatin is folded, or whether a change in folding would make a difference.

Not only did the MIT experiment have no effect on blood vessel growth, but it also failed to control tumors. The MIT researchers injected fibrosarcomas—one of the tumors used in the O'Reilly-Folkman mouse experiment—into mice in different ways to simulate local and metastasized tumors. Again, they found no difference between endostatin-treated mice and controls.

The second experiment reported in *Molecular Therapy*—run by a group at the British Columbia Cancer Agency in Vancouver, Canada, including Connie Eaves and Wolfgang Eisterer—took a similar approach. This group targeted a cancer of the blood, acute lymphocytic leukemia (ALL), for endostatin therapy. The Vancouver group withdrew ALL cells from four patients and implanted them into immune-deficient mice. With gene transfer, the researchers also got mice to express relatively high levels of endostatin—180 ng/ml—in the blood. But when they compared the endostatin-producing mice with controls, they found no difference in cancer burden.

Leboulch says his group took steps to see that the endostatin it produced was as close as possible to the original form. The researchers tested the protein produced by the transplanted gene to ensure that it inhibited endothelial cell proliferation, examined its amino acid sequence, and ran confirmatory antibody checks.

He also says an earlier version of their paper was turned down for publication by *Science* because it lacked a "positive control"—a substance illustrating effective tumor control to compare with the endostatin failures. To remedy this, Leboulch tried to repeat the original 1997 mouse experiments. Leboulch's postdoc, Bachelot, asked Folkman's group for samples of the original *E. coli* precipitate but never received any. So Bachelot made injectable endostatin using the original *E. coli* recipe. This also produced no effect.

Failed experiments such as these often don't get published, but Leboulch says he decided to submit the results partly because his ex-postdocs wanted this work to get out, and partly because "some of my colleagues at Harvard encouraged me to make the data available." One former Harvard researcher, asking not to be named, grumbles that he and "thousands of postdocs" have had the same disappointing experience. Although Leboulch admires Folkman and endorses his antiangiogenic program, he says: "We think we will get out of this endostatin business."

The beat goes on

The failure of these two experiments points up what Folkman calls "a paradox": Endostatin delivered to the body by gene therapy appears to be less effective than when the protein is simply injected. Last year, in a paper co-authored by Folkman in the *Proceedings of the National Academy of Sciences*, Richard Mulligan's group at Harvard compared the potency of five antiangiogenic compounds delivered by modified adenoviruses to mice. Ranked by efficacy, endostatin was at the bottom.

Quiet celebrity. Judah Folkman with Senator Edward Kennedy (D–MA) and on the cover of a recent book.

"The mechanism of this paradox is unknown," Folkman writes in a comment faxed to *Science*. The high concentrations of the protein produced by gene therapy, he speculates, might lead to "protein aggregation" that renders endostatin inactive. Mouse receptors might become overloaded at high serum concentrations, although the identity of the receptor is not known. And the gene-produced molecule might be more vulnerable to degradation or metabolic processes.

Yet even this paradoxical behavior is not consistent. Folkman notes that a gene therapy experiment by Andrew Feldman and Steven Libutti at NCI did produce some promising results. Feldman and Libutti transplanted an endostatin gene into mouse liver tumor cells and implanted the cells into mice. As they reported in the *Journal of the National Cancer Insitute* last year, the implants expressing the highest amounts of endostatin were most strongly inhibited from growing. Although Folkman speculates that high levels of endostatin may overload receptors, Libutti thinks that endostatin concentrations of 1 μg/ml or more—higher than described in either *Molecular Therapy* report—are needed locally to have an effect.

To O'Reilly, the fact that some groups have seen at least modest tumor inhibition in gene therapy experiments suggests a simple explanation for the failure of the two studies reported in *Molecular Therapy*: The proteins produced in both experiments were defective.

In contrast to gene therapy experiments, Folkman says protein-injection studies have yielded many positive reports. A recent one, co-authored by Folkman, Oliver Kisker, and other Harvard scientists in *Cancer Research* last October, reports "tumor regression" in immune-deficient mice treated with endostatin delivered continuously by a small implanted osmotic pump.

The researchers used a soluble, yeast-produced form of human recombinant endostatin, the same material that EntreMed gives patients in its clinical trials. They calculated that the minipumps delivered systemic doses of 200 to 300 ng/ml. Although this is lower than in the Leboulch gene therapy experiment, Folkman notes that this method of delivery was up to 10-fold "more effective" at controlling new blood vessels than periodic injections in most studies were—with the exception of the remarkable effects seen in the 1997 study.

O'Reilly agrees that it makes sense to investigate all of the discrepancies and puzzles in the results with endostatin so far. But he argues that these investigations should not hold up clinical trials, because "patients with advanced cancer are desperate" and "don't have the luxury of waiting." EntreMed has received clearance from the U.S. Food and Drug Administration to expand its clinical trials to investigate responses to different doses. Even Leboulch says that clinical trials are now likely to provide the best new information on whether endostatin really works.

–ELIOT MARSHALL

CREDITS: (TOP TO BOTTOM) DENNIS COOK/AP; RANDOM HOUSE

EXHIBIT C

Effect of Retroviral Endostatin Gene Transfer on Subcutaneous and Intraperitoneal Growth of Murine Tumors

Andrew L. Feldman, H. Richard Alexander, Stephen M. Hewitt, Dominique Lorang, Christina E. Thiruvathukal, Ewa M. Turner, Steven K. Libutti

Background: **Inhibiting tumor angiogenesis is a promising new strategy for treating cancer. Difficulties with the stability, manufacture, and long-term administration of recombinant antiangiogenic proteins have prompted investigators to use gene therapy to generate these proteins *in vivo*. We investigated whether transfer of the gene encoding the angiogenesis inhibitor endostatin into the murine liver cell line NMuLi could inhibit tumor growth *in vivo*. *Methods:* NMuLi cells were transduced with retroviral vectors containing the murine endostatin gene. The presence and function of endostatin in transduced cell supernatants were confirmed by competitive enzyme immunoassay and endothelial cell proliferation assays. Nude mice were given a subcutaneous or intraperitoneal injection with NMuLi cells, control transduced cells (NEF-null), or endostatin-transduced clones (NEF-Endo1 to 4) and were monitored for tumor growth. All statistical tests were two-sided. *Results:* Supernatants from the clone secreting the lowest amount of endostatin (NEF-Endo4, 28 ng/mL) inhibited endothelial cell proliferation by 6% (95% confidence interval [CI] = 0% to 12%), and those from the clone secreting the highest amount (NEF-Endo1, 223 ng/mL) inhibited endothelial cell proliferation by 20% (95% CI = 13% to 27%). Increased levels of endostatin were detected in tumor lysates, but not serum, of mice given a subcutaneous injection of NEF-Endo1 cells. After 63 days, mice given a subcutaneous injection of parental NMuLi or NEF-null cells had tumor volumes of 2400 mm^3 (95% CI = 1478 mm^3 to 3300 mm^3) and 2700 mm^3 (95% CI = 2241 mm^3 to 3144 mm^3), respectively, compared with mean tumor volumes of less than 30 mm^3 in mice given an injection of NEF-Endo clones, a statistically significant difference ($P<.001$). After 123 days, all 16 mice given an intraperitoneal injection of parental NMuLi or NEF-null cells had died, compared with only three (9%) of 32 mice given an injection of NEF-Endo clones. *Conclusions:* Retroviral endostatin gene transfer leads to secretion of functional endostatin that is sufficiently active to inhibit tumor growth. Further studies of retroviral endostatin gene transfer for the treatment of cancer are warranted. [J Natl Cancer Inst 2001;93: 1014–20]**

Because tumors require angiogenesis for sustained growth *(1)*, inhibiting tumor angiogenesis is a promising new strategy for treating cancer patients. However, difficulties in the stability, manufacture, and long-term administration of recombinant forms of endogenous antiangiogenic proteins have led investigators to develop gene therapy approaches to the antiangiogenic treatment of cancer *(2)*. Endostatin, a 20-kd C-terminal fragment of collagen XVIII *(3)*, is a potent antiangiogenic agent currently being evaluated in clinical trials *(4)*.[1] We tested whether retroviral transfer of the endostatin gene could generate sufficient functional endostatin *in vivo* to inhibit tumor growth.

Materials and Methods

Generation of Pseudotyped Retroviral Particles

The retroviral vector pCLNCX *(5)* and the vector pMD.G containing the G protein gene from vesicular stomatitis virus (VSV), as well as the cell line 293GP stably transfected with the retroviral gag and pol elements, were obtained from P. Robbins, National Cancer Institute (NCI), Bethesda, MD. The human elongation factor (EF) 1α promoter was isolated from the plasmid pAd.EF1 (Z. Guo, NCI) by *Bam*HI/*Hin*dIII digestion (New England Biolabs Inc., Beverly, MA) and ligated in place of the downstream cytomegalovirus (CMV) promoter in *Bam*HI/*Hin*dIII-digested pCLNCX to generate the vector pEF-null (Fig. 1, a). Generation of the construct ss-mEndo, consisting of the 18-amino acid E3/19K signal sequence followed by the murine endostatin gene cloned from murine liver, has been described previously *(6)*. The ss-mEndo construct was cloned into *Hin*dIII/*Cla*I-digested pEF-null to generate the vector pEF-Endo or into *Hin*dIII/*Cla*I-digested pCLNCX to generate the vector pCMV-Endo.

293GP cells were maintained in complete medium consisting of Dulbecco's modified Eagle medium (DMEM) with 10% fetal calf serum (FCS), 100 U/mL of penicillin, 100 μg/mL of streptomycin, 50 μg/mL of gentamicin, 0.5 μg/mL of Fungizone, and 4 m*M* glutamine (Biofluids, Rockville, MD). Cells were plated in 10-cm tissue culture dishes at a density of 3 × 10^6 cells/dish and allowed to adhere overnight in a 5% CO_2 incubator at 37 °C. Cells then were rinsed with phosphate-buffered saline (PBS), cotransfected with 6 μg of retroviral plasmid and 6 μg of pMD.G (for VSV envelope protein expression) by use of 60 μL of Lipofectamine (Life Technologies, Inc. [GIBCO BRL], Rockville, MD) in 6 mL of serum-free DMEM, and incubated at 37 °C for 3 hours. After the addition of 12 mL of complete medium, cells were further incubated for 24 hours before the medium was replaced with 10 mL of fresh complete medium. After 24 hours, the supernatant containing the retroviral particles was collected, passed through a 0.45-μm (pore size) filter, and stored at −70 °C until ready for use.

Retroviral Transduction of NMuLi Cells

The cell line NMuLi, an epithelioid nonparenchymal cell line derived from the NAMRU mouse liver *(7)*, was obtained from the American Type Culture Collection (Manassas, VA) and passaged in complete medium. We confirmed its ability to form malignant tumors in nude mice *(8)*. For the retroviral transduction, cells were plated in six-well tissue culture plates at a density of 100 000 cells/well and allowed to adhere overnight. The medium then was replaced with 1 mL of fresh complete medium, 1 mL of retroviral supernatant, and 8 μg/mL of hexadimethrine bromide (Sigma Chemical Co., St. Louis, MO) to assist the uptake of viral particles. After incubation at 37 °C for 4 hours, the medium was replaced with 2 mL of fresh complete medium. The transduction was repeated the following day. Because the retroviral vector contains a selectable marker, the neomycin resistance gene, G418 (400 μg/mL; Life Technologies, Inc.), was added to the medium 24 hours after the second transduction. The surviving G418-resistant cells were amplified. To obtain individual clones, the endostatin-transduced cells (NEF-Endo) were plated in limiting dilution in complete medium containing G418 (400 μg/mL). The resultant clones were isolated by use of 8-mm diameter cloning cylinders (Specialty Media, Phillipsburg, NJ) and amplified.

To confirm endostatin expression, parental NMuLi cells, pEF-null-transduced cells (NEF-null), or NEF-Endo clones were plated in six-well plates at a density of 10^6 cells/well and allowed to adhere overnight. The medium was replaced with 1 mL/well of modified complete medium containing 5% FCS and incubated at 37 °C for 24 hours. Cell su-

Affiliations of authors: A. L. Feldman, H. R. Alexander, D. Lorang, C. E. Thiruvathukal, E. M. Turner, S. K. Libutti (Surgical Metabolism Section, Surgery Branch, Center for Cancer Research), S. M. Hewitt (Laboratory of Pathology, Center for Cancer Research), National Cancer Institute, Bethesda, MD.

Correspondence to: Steven K. Libutti, M.D., National Institutes of Health, Bldg. 10, Rm. 3C428, Bethesda, MD 20892 (e-mail: Steven_Libutti@nih.gov).

See "Notes" following "References."

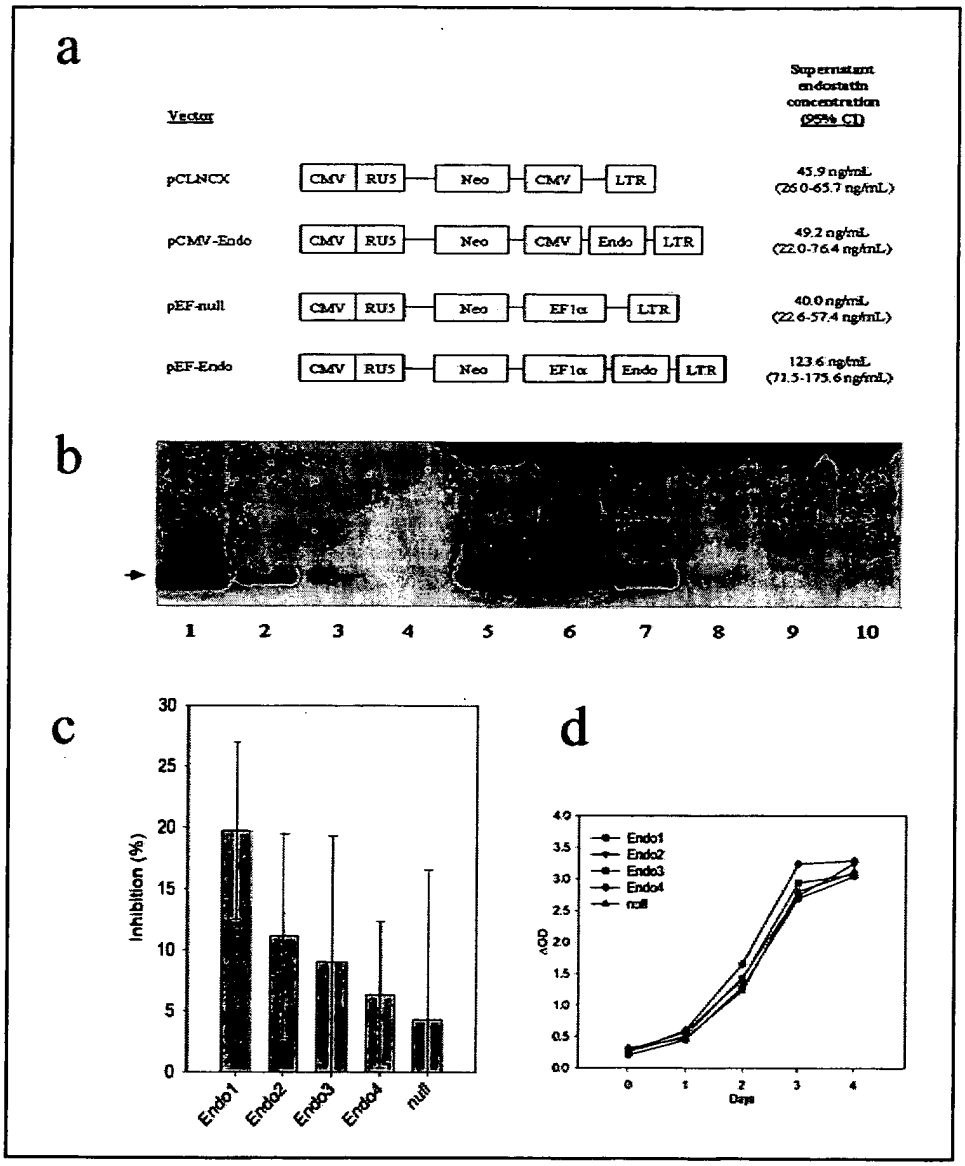

Fig. 1. Production and *in vitro* characteristics of retrovirally transduced NMuLi cells. **Panel a:** schematic representations of the retroviral vectors used in this study, with the supernatant endostatin concentrations, determined by competitive enzyme immunoassay (EIA), resulting from transduction of NMuLi cells and selection of G418-resistant cell populations (**right**). The murine endostatin gene was cloned into pCLNCX *(5);* however, NMuLi transduction resulted in minimal increases of supernatant endostatin levels above baseline. Substitution of the downstream cytomegalovirus (CMV) enhancer promoter with the human elongation factor (EF) 1α promoter yielded substantial elevation of the supernatant endostatin concentration. **Panel b:** western blot demonstrating secreted endostatin protein in transduced cell culture supernatants after 24 hours. **Arrow at left** denotes 20-kd molecular mass. **Lanes 1, 2,** and **3**—recombinant murine endostatin at concentrations of 500, 125, and 31 ng/mL, respectively; **lane 4**—water; **lane 5**—NEF-Endo1 supernatant (endostatin-competitive EIA concentration, 223 ng/mL); **lane 6**—NEF-Endo2 supernatant (endostatin-competitive EIA concentration, 163 ng/mL); **lane 7**—NEF-Endo3 supernatant (endostatin-competitive EIA concentration, 42 ng/mL); **lane 8**—NEF-Endo4 supernatant (endostatin-competitive EIA concentration, 28 ng/mL); **lane 9**—NEF-null supernatant (endostatin-competitive EIA concentration, 20 ng/mL); and **lane 10**—parental NMuLi supernatant (endostatin-competitive EIA concentration, 4 ng/mL). **Panel c:** The ability of culture supernatants from transduced cells to inhibit bovine capillary endothelial cell proliferation was measured after 72 hours by the WST-1 colorimetric assay. Inhibition is compared with unconditioned medium containing 1 ng/mL of basic fibroblast growth factor. Inhibition by NEF-Endo1 supernatant was statistically significantly higher than inhibition by NEF-null supernatant (P = .037), and there was a statistically significant trend of increasing inhibition with increasing endostatin concentration (P=.01). **Panel d:** *In vitro* proliferation of transduced cells was measured daily by the WST-1 colorimetric assay. Results are expressed as the change in optical density (ΔOD) at 450 nm after incubation with the tetrazolium salt WST-1. Each **data point** represents the mean value of eight identical wells. *In vitro* growth characteristics were similar among the transduced cell lines. CI = confidence interval.

pernatants then were collected and passed through a 0.45-μm (pore size) filter. Endostatin concentrations in the supernatants were determined by competitive enzyme immunoassay (EIA) (Cytimmune Sciences, College Park, MD) according to the instructions of the manufacturer. Four endostatin-transduced clones with varying supernatant endostatin concentrations were selected for further study and designated NEF-Endo1 to 4, in decreasing order of endostatin production. The molecular weight of endostatin was determined from the culture supernatants by western blotting (NuPAGE; Novex, San Diego, CA) by use of 570 ng/mL of rabbit antimurine endostatin polyclonal immunogloblin G antibody (from Cytimmune Sciences). The EIA murine endostatin standard was used as a positive control.

Functional Assay of Retrovirally Derived Endostatin

To assess the functional activity of the retrovirally derived endostatin, we tested the culture supernatants in endothelial cell proliferation assays as described previously *(6),* with slight modifications. Briefly, bovine adrenal capillary endothelial cells (EJG; American Type Culture Collection) were plated in 200 μL of complete medium in collagen I-coated 96-well plates (Biocoat; Becton Dickinson Labware, Bedford, MA) at a density of 1000 cells/well and were incubated overnight at 37 °C. The medium then was aspirated and replaced with 100 μL of cell supernatant or modified complete medium containing 5% FCS. Basic fibroblast growth factor (R&D Systems, Inc., Minneapolis, MN) at a final concentration of 1 ng/mL was added to all wells as a stimulus of proliferation. After incubation at 37 °C for 72 hours, proliferation was analyzed by the WST-1 assay (Roche, Indianapolis, IN) according to the manufacturer's instructions. The inhibition of proliferation by each sample was calculated according to the formula: inhibition (%) = (mean ΔOD_{media} − ΔOD_{sample}/mean ΔOD_{media}) × 100, where ΔOD represents the change in optical density at 450 nm (Multiskan MCC/340 plate reader; Titertek, Huntsville, AL) from before the addition of the WST-1 reagent to the completion of the 4 hours' incubation at 37 °C. Each sample was measured in six individual wells. The experiment was repeated to confirm results.

In Vitro Growth of Parental and Transduced NMuLi Cells

To place in perspective any differences noted in the *in vivo* growth of parental and transduced cell lines, we compared *in vitro* growth rates. Five identical 96-well tissue culture plates were prepared. On each plate, NMuLi, NEF-null, and NEF-Endo1 to 4 cells were each plated in eight identical wells at a density of 1000 cells/well in 100 μL of complete medium. WST-1 proliferation assays were performed daily on days 0–4, as described above. Cells were allowed to adhere for 3 hours before the day-0 assay was performed. Proliferation was expressed as the ΔOD as defined above.

Tumor Formation by Retrovirally Transduced Cells

Animal experiments were conducted according to protocols approved by the National Institutes of Health Animal Care and Use Committee (Bethesda, MD). Eight-week-old female nude mice (Charles River Laboratories, Wilmington, DE) were given an injection of 5×10^5 parental NMuLi cells, NEF-null cells, or NEF-Endo1 to 4 cells. Subcutaneous injections were administered in the right flank in 100 μL of PBS. Tumors were measured in two dimensions by use of calipers at regular intervals, and tumor volumes were calculated according to the following formula: volume = width^2 × length × 0.52, where 0.52 is a constant to calculate the volume of an ellipsoid. Intraperitoneal injections were administered in 2 mL of PBS. All animals were followed for survival. Each group consisted of eight mice.

Measurement of Endostatin Levels *In Vivo*

Mice were given an inoculation of NEF-null or NEF-Endo1 cells subcutaneously or intraperitoneally as described above. Serum samples obtained 45 days (subcutaneous model) or 21 days (intraperitoneal model) after tumor cell inoculation were analyzed for endostatin concentration by EIA. Serum samples also were obtained from non-tumor-bearing animals. Each group consisted of six mice.

To assess *in vivo* local endostatin expression, we harvested NEF-null or NEF-Endo1 tumors immediately after the mice were killed 38 days after subcutaneous injection as described above. Tumors were snap-frozen in liquid nitrogen and stored at −80 °C until ready for analysis. Tumors then were homogenized at room temperature in a protease inhibitor cocktail (Complete Mini; Roche) dissolved in 10 m*M* HEPES and 1 m*M* EDTA by use of a bead homogenizer (FastPrep; Savant Instruments, Holbrook, NY). Homogenates were centrifuged at 7500*g* for 5 minutes at 4 °C, and supernatants were analyzed for endostatin by EIA. Endostatin concentrations were normalized to total protein concentrations determined by use of a BCA protein assay kit (Pierce Chemical Co., Rockford, IL) and a linear bovine serum albumin standard curve, according to the manufacturer's instructions.

Immunohistochemistry and Histopathologic Evaluation

NEF-null or NEF-Endo1 tumors were harvested immediately after the mice were killed 38 days after subcutaneous injection, as described above. Tumors were snap-frozen in liquid nitrogen and stored at −80 °C. Frozen sections (7 μm) were cut on a cryostat and stained with hematoxylin–eosin (H&E) or antibodies specific for CD31 (PharMingen, San Diego, CA), proliferating cell nuclear antigen (PCNA) (Zymed Laboratories, San Francisco, CA), or caspase-3 (PharMingen). For immunostaining, sections were fixed in acetone for 10 minutes (CD31) or in 4% formalin for 1 hour (PCNA and caspase-3). After endogenous peroxidase activity was blocked by use of 3% hydrogen peroxide in methanol for 10 minutes, sections were incubated for 1 hour in a blocking solution containing 10% normal goat serum. Sections were incubated with primary antibody at 4 °C overnight (CD31 at 1 : 50 dilution or caspase-3 at a concentration of 1 μg/mL) or at room temperature for 1 hour (PCNA at 1 : 50 dilution). Slides were then washed three times in PBS, incubated in biotinylated species-specific appropriate secondary antibody for 1 hour, and exposed to avidin–biotin–peroxidase complex (Vector Laboratories, Inc., Burlingame, CA). Sections were reacted with 0.06% 3,3′-diaminobenzidine (Sigma Chemical Co.) and counterstained with hematoxylin.

H&E and immunostained sections were analyzed by a pathologist (S. M. Hewitt), who was blinded to the identity of the groups. Only good-quality sections with uniform, well-demarcated staining and low background were analyzed. Microvascular density of each tumor was assessed by use of a scoring system (Table 1) based on the mean number of CD31-positive cells per high-power field (hpf, ×600). Proliferative and apoptotic cells were analyzed by use of a similar scoring system (Table 1) based on the number of cells per hpf staining positively for PCNA and caspase-3, respectively.

Statistical Analysis

Data are presented as means with 95% confidence intervals (CIs). Comparisons between groups were made by use of the Mann–Whitney *U* test or the Kruskal–Wallis test, where appropriate. The endostatin dose dependence of endothelial cell inhibition was analyzed by use of the Jonckheere–Terpstra trend test. Two-tailed *P* values <.05 were considered to be statistically significant.

RESULTS

In Vitro Production of Endostatin

Initial transduction of NMuLi cells with pCMV-Endo did not yield supernatant endostatin concentrations above baseline. We, therefore, replaced the downstream CMV promoter in pCLNCX with the EF1α promoter (Fig. 1, a) based on favorable results reported with the use of the EF1α promoter in other viral gene delivery systems *(9,10)*. Endostatin levels in supernatants from NEF-Endo1, 2, 3, and 4, NEF-null, and NMuLi cells were 223, 163, 42, 28, 20, and 4 ng/mL per 10^6 cells, respectively. The NEF-Endo clone supernatants produced specific 20-kd bands on western blot proportional in intensity to the endostatin concentration as measured by EIA (Fig. 1, b).

In Vitro Function of Endostatin

To determine if the endostatin produced by the transduced clones was biologically active, we tested the supernatants in an endothelial cell proliferation assay. Compared with unconditioned medium, supernatants from the transduced clones NEF-Endo 1, 2, 3, and 4, and NEF-null inhibited endothelial cell proliferation by 20% (95% CI = 13% to 27%), 11% (95% CI = 3% to 19%), 9% (95% CI = −1% to 19%), 6% (95% CI = 0% to 12%), and 4% (95% CI = −8% to 17%), respectively (Fig. 1, c). The difference in inhibition between supernatants from NEF-Endo1 and NEF-null was statistically significant (P = .037). The dose-dependent inhibition of endothelial cell proliferation by endostatin also was statistically significant (P = .01).

In Vitro Growth of Parental and Transduced NMuLi Cells

We next compared the *in vitro* growth of the transduced cells with that of the

Table 1. Histopathologic findings in transduced NMuLi tumors*

	Diameter, mm	CD31†	Caspase-3‡	PCNA‡
Endostatin transduced				
Tumor 1	1.75	3	1	4
Tumor 2	1.75	3	1	1
Tumor 3	2.00	4	2	4
Tumor 4	2.00	3	1	4
Tumor 5	1.50	3	0	2
Tumor 6	1.25	2	n.d.	3
Mean	1.71	3	1	3
95% CI	1.41 to 2.01	2.3 to 3.7	0.1 to 1.9	1.7 to 4.3
Null transduced				
Tumor 1	4.00	4	2	4
Tumor 2	5.00	4	2	4
Tumor 3	5.50	4	3	4
Tumor 4	5.00	4	2	4
Mean	4.88	4	2.3	4
95% CI	3.87 to 5.88	—	1.5 to 3.0	—

*PCNA = proliferating cell nuclear antigen; n.d. = not determined; CI = confidence interval.

†CD31 scoring system defined on the basis of immunohistochemical staining of tumor tissue sections: 0 = <1 cell/high-power field (hpf); 1 = 1–5 cells/hpt; 2 = 6–10 cells/hpf; 3 = 11–15 cells/hpf; 4 = >15 cells/hpf.

‡Caspase-3 and PCNA scoring system defined on the basis of immunohistochemical staining of tumor tissue sections: 0 = no cells/hpf; 1 = <1 cell/hpf; 2 = 1–2 cells/hpf; 3 = 2–3 cells/hpf; 4 = >3 cells/hpf.

parental NMuLi cells. All four NEF-Endo clones demonstrated similar patterns of *in vitro* growth to each other and to NEF-null cells (Fig. 1, d). Parental NMuLi cells grew more slowly *in vitro* than their transduced, G418-selected counterparts.

Subcutaneous Growth of Parental and Transduced NMuLi Cells

Mice given a subcutaneous injection of NMuLi or NEF-null cells developed rapidly growing tumors and were killed 63 days after injection when the tumor volumes were 2400 mm^3 (95% CI = 1478 mm^3 to 3300 mm^3) and 2700 mm^3 (95% CI = 2241 mm^3 to 3144 mm^3), respectively (Fig. 2, a). The mean tumor volumes were less than 30 mm^3 in all four groups given an injection of NEF-Endo clones (P.<001 versus null-transduced tumors). Although the largest endostatin-transduced tumors were observed in the mice that were given an injection of the NEF-Endo4 clone, which produced the lowest amount of endostatin *in vitro* (NEF-Endo4; Fig. 2, a, inset), the difference between the size of the endostatin-transduced tumors was not statistically significant (P = .52 at day 63). After 122 days of follow-up, mice given an injection of NEF-Endo clones 1, 3, and 4 had tumor volumes of 70 mm^3 (95% CI = 0 mm^3 to 202 mm^3), 300 mm^3 (95% CI = 123 mm^3 to 419 mm^3), and 90 mm^3 (95% CI = 0 mm^3 to 279 mm^3), respectively. Mice given an injection of the NEF-Endo clone 2 had nonpalpable or barely palpable lesions.

Intraperitoneal Growth of Parental and Transduced NMuLi Cells

Mice given an intraperitoneal injection of parental NMuLi or NEF-null cells had median survival times of 58 days (95% CI = 49 days to 61 days) and 56 days (95% CI = 46 days to 92 days), respectively, and all mice were dead by day 123 (Fig. 2, b). At this time, only three (9%) of the 32 mice receiving intraperitoneal NEF-Endo clones had died. Autopsies of mice that died revealed massive peritoneal tumor deposits and ascites. Surviving mice were killed and had an autopsy, revealing only occasional, small peritoneal deposits.

In Vivo Endostatin Levels

Serum endostatin levels were similar in mice bearing subcutaneous NEF-Endo1 tumors (mean, 33.8 ng/mL; 95% CI = 18.5 ng/mL to 49.1 ng/mL) and NEF-null tumors (mean, 32.6 ng/mL; 95% CI = 15.8 ng/ml to 49.4 ng/mL), respectively (P = .87). Serum endostatin levels in mice bearing intraperitoneal NEF-Endo1 tumors (mean, 40.0 ng/mL; 95% CI = 7.8 ng/mL to 72.2 ng/mL) were slightly higher than those in mice bearing intraperitoneal NEF-null tumors (mean, 22.2 ng/mL; 95% CI = 18.3 ng/mL to 26.1 ng/mL), but this difference was not statistically significant (P = .42). Serum endostatin levels in non-tumor-bearing mice were 27.1 ng/mL (95% CI = 15.3 ng/mL to 38.9 ng/mL), similar to

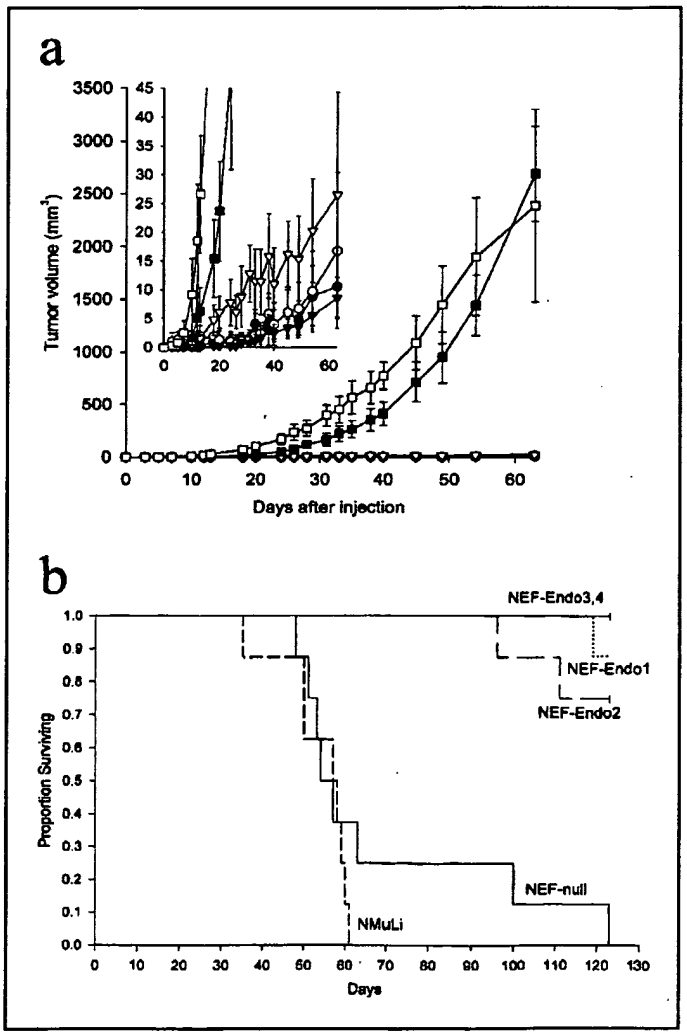

Fig. 2. *In vivo* characteristics of retrovirally transduced NMuLi cells. **Panel a:** assessment of tumor volume in mice given a subcutaneous injection of 5 × 10^5 parental NMuLi cells (□), NEF-null cells (■), or NEF-Endo clones 1 (●), 2 (○), 3 (◀), and 4 (◁). **Each point** represents the mean volume of eight mice. **Inset** shows expanded *y*-axis to demonstrate tumor volumes of NEF-Endo clones. Control (NMuLi and NEF-null) mice were killed on day 63, at which time the mean tumor volumes among NEF-Endo groups were less than 30 mm^3. **Panel b:** mouse survival after intraperitoneal inoculation of parental and transduced NMuLi cells. Each group contained eight mice. Median survival times were 58 days for mice receiving NMuLi cells and 56 days for mice receiving NEF-null cells. All control animals died by day 123, at which time only three (9%) of the 32 animals receiving NEF-Endo clones had died. The numbers of mice at risk in each group after 60 days were as follows: NMuLi = 2 (95% confidence interval [CI] = 0.2 to 3.8); NEF-null = 3 (95% CI = 0.6 to 4.7); NEF-Endo1 = 8 (95% CI = 5.0 to 8.0); NEF-Endo2 = 8 (95% CI = 5.0 to 8.0); NEF-Endo3 = 8 (95% CI = 5.0 to 8.0); and NEF-Endo4 = 8 (95% CI = 5.0 to 8.0). The numbers of mice at risk in each group after 120 days were as follows: NMuLi = 0; NEF-null = 1 (95% CI = 0.0 to 4.2); NEF-Endo1 = 7 (95% CI = 4.2 to 7.8); NEF-Endo2 = 6 (95% CI = 3.3 to 7.4); NEF-Endo3 = 8 (95% CI = 5.0 to 8.0); and NEF-Endo4 = 8 (95% CI = 5.0 to 8.0).

values we reported previously *(11)* and not statistically significantly different from any of the other groups tested.

To confirm endostatin production in the NEF-Endo tumors, we measured local endostatin levels in tumor lysates 38 days after subcutaneous injection. Tumor lysates from endostatin- and null-transduced tumor had 38.7 ng (95% CI = 20.7 ng to 56.6 ng) and 11.2 ng (95% CI = 8.8 ng to 13.7 ng) endostatin/mg total protein, respectively (P = .006).

Immunohistologic Characteristics of Transduced Tumors

Evaluation of H&E-stained tumor sections revealed small tumor nodules (mean diameter, 1.7 mm) derived from NEF-Endo1 and NEF-Endo2 cells (Table 1; Fig. 3, a); tumors derived from NEF-null cells were larger (mean diameter, 4.9 mm) and had zones of central necrosis (Fig. 3, b). Mean microvessel density scores for endostatin- and null-transduced tumors were 3 and 4, respectively (Table 1; Fig. 3, c–e). Endostatin-transduced tumors did not have discernible vessels on H&E stains (Fig. 3, a) but did contain CD31-positive endothelial cells as individual capillaries (Fig. 3, c). By contrast, the null-transduced tumors contained easily identifiable vessels on H&E stains, away from zones of necrosis (Fig. 3, b). The CD31 staining highlighted larger vessels as well as individual endothelial cells (Fig. 3, d).

Finally, we determined the apoptotic and proliferative scores for the endostatin- and null-transduced tumors. The null-transduced tumors had higher apoptotic and proliferative scores than the endostatin-transduced tumors, with mean apoptosis scores for endostatin- and null-transduced tumors of 1 and 2.3, respectively, and mean proliferative scores of 3 and 4, respectively (Table 1; Fig. 3, e).

Discussion

The demonstration that tumors require neoangiogenesis for sustained growth *(1)* has prompted intense investigation into the inhibition of tumor angiogenesis as a strategy for treating cancer patients. More than 40 endogenous inhibitors of angiogenesis have been described previously *(2)*. Of interest, some of these inhibitors are tumor derived, including endostatin, a 20-kd C-terminal fragment of collagen XVIII originally isolated from a murine hemangioendothelioma cell line *(3)*. There are, however, a number of potential limitations to treating cancer with antiangiogenic agents, such as endostatin, in their recombinant forms. First, some of these recombinant biologics, including endostatin, are relatively unstable *in vitro* and require high doses for antitumor efficacy *(3)*. Second, many of these agents are not cytotoxic to tumor cells themselves and may need to be administered chronically *(12)*. Finally, systems that allow continuous delivery of antiangiogenic agents appear to be therapeutically preferable to the peak/trough kinetics associated with bolus infusion of recombinant proteins *(13,14)*. These limitations have prompted investigators to study gene therapy approaches to deliver antiangiogenic proteins for the treatment of cancer *(2)*.

Previously, we and others *(6,15,16)* have shown that adenoviral transfer of the endostatin gene to mice leads to high circulating endostatin levels that inhibit tumor growth. Several groups *(17–19)* also have demonstrated that nonviral endostatin gene transfer can cause an antitumor effect in mice. Both of these approaches, however, are associated with limitations in their ability to be translated for the treatment of human patients: The use of adenoviral vectors is limited because of immunogenicity and toxicity, and nonviral vectors generally are associated with low levels of transgene expression. Because retroviruses have been used safely in human gene therapy trials *(20,21)*, we investigated whether retrovi-

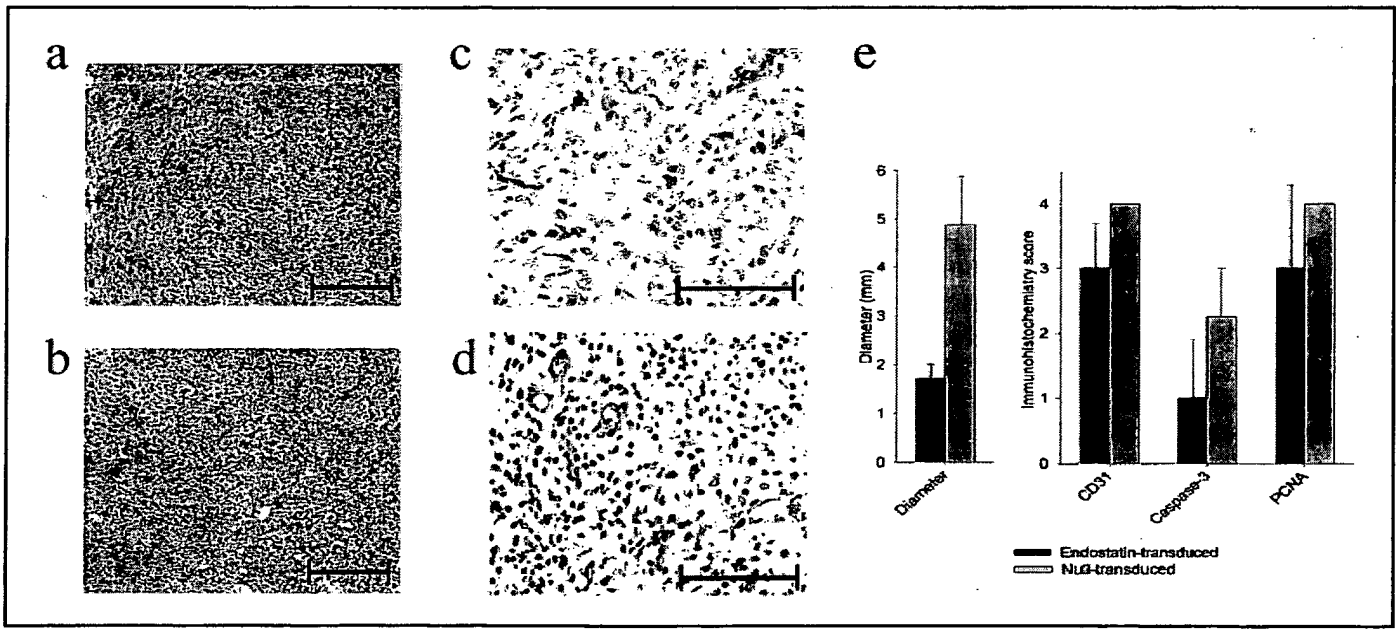

Fig. 3. Histopathologic findings in transduced NMuLi tumors. **Panel a:** representative section from an endostatin-transduced tumor (hematoxylin–eosin [H&E], original magnification ×200; **scale bar** = 50 μm). **Panel b:** representative section from a null-transduced tumor (H&E, original magnification ×200; **scale bar** = 50 μm). **Panel c:** microvessel staining from a representative section of an NEF-Endo1 tumor (anti-CD31 immunostaining, original magnification ×400; **scale bar** = 25 μm). **Panel d:** microvessel staining from a representative section of an NEF-null tumor (anti-CD31 immunostaining, original magnification ×400; **scale bar** = 25 μm). **Panel e:** Mean tumor diameters and scores for CD31, caspase-3, and proliferating cell nuclear antigen (PCNA) immunostaining in endostatin- and null-transduced tumors.

ral transfer of the endostatin gene to tumor cells could result in sufficient functional protein to inhibit tumor growth *in vivo*.

Because endostatin occurs naturally as a cleavage product of collagen XVIII, the coding sequence for endostatin lacks a secretion signal. We previously generated a construct in which the endostatin coding sequence is preceded by the adenoviral protein E3/19K signal sequence *(6,22)*. We cloned this construct into the multiple cloning site of pCLNCX, a Moloney murine leukemia virus-derived retroviral expression plasmid *(5)*, and generated pseudotyped retroviral particles containing the VSV glycoprotein G *(23)*. NMuLi cells transduced with this construct were resistant to G418, but they did not generate high levels of endostatin in their cell supernatants. We hypothesized that this was a promoter-related phenomenon and replaced the downstream CMV promoter in the pCLNCX plasmid with the constitutively active human EF-1α promoter. Cells transduced with this modified construct secreted high endostatin levels, as measured in a competitive EIA.

To verify that the secreted protein measured in the EIA was, in fact, endostatin, we performed western blotting of aliquots from supernatants of the transduced cells. Supernatants from NEF-Endo clones produced bands of equal mobility to that of recombinant murine endostatin and of intensities proportional to the EIA-measured endostatin concentrations. When these supernatants were placed on bovine adrenal capillary endothelial cells, they inhibited endothelial cell proliferation in a dose-dependent fashion, confirming the activity of the secreted endostatin protein. The secreted endostatin was relatively endothelial cell specific, as has been reported previously *(3)*, because the growth rates of the NEF-null cells and the NEF-Endo clones were similar, including NEF-Endo1 cells that secreted the highest level of endostatin.

By contrast with the *in vitro* growth, transduction of NMuLi cells with the endostatin gene profoundly inhibited their subcutaneous growth in mice. Consistent with the antiangiogenic function of endostatin, NEF-Endo cells were capable of forming tumors *in vivo*, although these tumors were very small and displayed fewer microvessels than their null-transduced counterparts. The NEF-Endo4 clone, which produced the least endostatin *in vitro*, initially produced larger tumors than the other NEF-Endo clones tested. However, the inhibition of *in vivo* tumor growth of all clones was not directly proportional to *in vitro* endostatin expression. In fact, the growth curves of the four NEF-Endo clones began to diverge after approximately 100 days, although tumors in all groups remained much smaller than tumors in the NEF-null mice, which exceeded 2 cm^3. The eventual divergence of the growth curves among the NEF-Endo clones growth may reflect clonal variations unrelated to endostatin. Alternatively, local endostatin production at late time points *in vivo* may not correspond with *in vitro* expression by various clones.

Of interest, endostatin transduction of NMuLi cells also inhibited their ability to form aggressive lesions in the peritoneal cavity. Like tumors in other sites, the pathogenesis of tumors of the peritoneal cavity has been shown to be associated with angiogenesis *(1,24,25)*. In addition, several inhibitors of angiogenesis have been reported to inhibit peritoneal tumor growth in rodent models, including protamine *(26)*, TNP-470 *(27–29)*, and agents that inhibit the action of vascular endothelial growth factor *(30–32)*. Our data suggest that endostatin also can inhibit peritoneal tumor growth. Similar to the results seen with our subcutaneous model, the tumor lesions present in mice receiving intraperitoneal NEF-Endo cells were generally small. In mice with subcutaneous or intraperitoneal tumors, circulating endostatin levels were not statistically significantly increased in mice harboring endostatin-transduced tumors. Because of the minimal amount of tumor tissue in these animals and the baseline mean serum endostatin concentration of 27 ng/mL, small amounts of endostatin released into the circulation may not have been detectable by the assay employed. The assay was, however, sensitive enough to detect increased concentrations of endostatin in lysates of endostatin-transduced tumors, confirming that endostatin was being produced locally in these animals.

Our results indicate that retroviral transfer of the endostatin gene to eukaryotic cells does not inhibit their growth *in vitro* but generates a secreted, functional endostatin protein that inhibits endothelial cells *in vitro* and tumor formation at multiple sites *in vivo*. In addition, these data support previous evidence *(26–32)* that inhibiting angiogenesis is a promising strategy for treating tumors of the peritoneal cavity. However, one limitation of this study is that *ex vivo* transduction of tumor cells is not a strategy applicable to clinical therapeutic use. Recently, retroviruses secreting another antiangiogenic molecule, interleukin 12 (IL-12), have been used in preclinical models of disseminated peritoneal cancer. Sanches et al. *(33)* transduced fibroblasts with the IL-12 gene *ex vivo*, then introduced them into mice with peritoneal ovarian carcinomatosis. Lechanteur et al. *(34)* used an intraperitoneal injection of retroviral packaging cells carrying the IL-12 gene as part of a combined approach to treating peritoneal colon carcinomatosis in rats. In each case, at least part of the tumor response was because of immune (i.e., nonantiangiogenic) mechanisms, and the immune effects of IL-12 have been associated with considerable toxicity in clinical and preclinical models. By contrast, endostatin appears to impart its antitumor effect solely by inhibiting angiogenesis, with minimal toxicity*(3,35)*. Therefore, further studies using retroviral endostatin gene transfer for the treatment of cancer, including disseminated tumors of the peritoneal cavity, are warranted.

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NOTES

[1]An overview of angiogenesis inhibitors in clinical trials is available from URL: http://cancertrials.nci.nih.gov/news/angio/table.html (accessed April 26, 2001).

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